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Chamblee, Georgia 30341

HUMAN HEALTH ASPECTS OF DDT

Statement by Homer R. Wolfe
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To DDT Hearing Officer, Washington State
Department of Agriculture

The effect of pesticides on man, including DDT, has been studied for a number of years. Much of the early Federal research, starting about 1949, was under what was then known as the Toxicology Program of the U. S. Public Health Service. Later the group was enlarged as the Pesticides Program under the National Communicable Disease Center activities; and more recently the pesticide research activities have been a responsibility of the Food and Drug administration. Our laboratory at Wenatchee, known as the Wenatchee Research Section laboratory, is one of the Federal laboratories under the Food and Drug Administration.

Our organization has conducted research with all of the major chlorinated hydrocarbon compounds. There has, however, been more research with DDT than with any of the compounds in that class. This, of course, is primarily because of the wide usage of DDT in agriculture and public health work over the last 25 years. More of the general population has undoubtedly been exposed, at least to some degree, to this compound than to any of the other so-called newer synthetic pesticides. The relatively thorough study of this compound is important because the similarity of action between the various chlorinated hydrocarbon compounds indicates that much of what has been learned about DDT should also be applicable, at least qualitatively, to related compounds.

While acutely DDT has relative low toxicity, what we are concerned with in this hearing today is not necessarily the acute effect of DDT on man but the effect of long-term exposure to low levels of the compound found in the environment.

In attempting to determine any adverse effects to the general population that may result from prolonged exposure to very small amounts of DDT in the environment or in the daily diet, it is well to keep in mind certain basic principles of pharmacology and toxicology. The dosage-response principle describes the relationship between quantity of a drug or chemical adsorbed and the effect observed. With rare exceptions, any effect of

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repeated exposure would appear more promptly, more frequently, more diversely, and more severely among persons whose exposure has been long and intensive.

Although the general population may have a prolonged exposure to DDT, the exposure is certainly not intense. Thus, any in-depth study of such a general population group most certainly would not be expected to show adverse effects anywhere near as soon as in a selected high exposure group. The most logical approach in an effort to obtain results within a reasonable length of time would, therefore, be to study people with relatively heavy and prolonged occupational exposure such as pesticide applicators or workers in pesticide formulating or manufacturing plants. Another type of useful study is of volunteers who agree to take known doses of pesticide over a long period of time and to undergo specific tests during this period.

The report summarizes in which

In our research DDT has been studied in both heavily exposed workers and in groups of volunteers given oral dosages of the pesticide.

The population is exposed to DDT from many sources, such as food, household uses, dust, and air. The total exposure is not known nor is the relative contribution from the various sources. The exposure from food has been, and continues to be, carefully investigated. The calculated intake of dietary DDT has been quite small with an average of slightly less than 0.2 mg. per man per day even when the use of DDT was at its peak. Present dietary levels based on total diet surveys, however, appear to be approximately 0.02 mg. per man per day, or about 1/10 of the earlier value. These surveys contain the kinds and quantities of foods consumed by the 16- to 19-year-old group in our population and represents almost twice the average dietary intake of the average individual.

It has been known for many years that DDT is stored in the fatty tissue. Results of our research, along with studies by others in the United States, show that this pesticide is found in practically everyone in the general population of this country. The first general survey for DDT was carried out by Laug and his colleagues in 1950 (Laug et al., 1951). They demonstrated DDT residues ranging from 0 to 34 ppm (mean, 5.3 ppm) in the fat of 75 persons who had no special occupational exposure.

In our studies during past years the mean storage level in the fatty tissue of the general population was found to be around 5 to 6 ppm for DDT and 6 to 7 ppm for metabolites of this compound--primarily DDE. The average amount of all DDT-derived material found was about 11 to 12 ppm. The mean storage level in later studies was found to be about 3 ppm for DDT and about 8 ppm for DDE, giving a total of 11 ppm of DDT-derived material. More recent studies by the State Services Pesticide Monitoring Group within the Food and Drug Administration showed a mean storage level of approximately 8 ppm for DDT-derived material.

Some of the earliest DDT storage studies in the United States did not include determination of DDE content. Even though the metabolite DDE is considerably less toxic to mammals than DDT it was felt that all DDT-derived material should be reported in the surveys made in later years. It is my understanding that because of this there has been some misinterpretation by the public of DDT storage findings. It is understandable why someone could be misled into believing there had been a great increase in storage in recent years if they had noted a 5 ppm value of DDT storage in an earlier study and 11 to 12 ppm for DDT-derived material in later studies, when, in fact, there hasn't appeared to be an increase in the trend of storage in fatty tissue of the general population of this country since the earliest survey in 1950.

The concern is, of course, whether or not DDT storage levels of the magnitude found are harmful to man. In this regard studies of men fed known daily doses of DDT and men with prolonged occupational exposure to DDT were carried out. In two study groups involving oral dosage a total of 90 men were involved (Hayes et al., 1956; Hayes et al., manuscript in preparation). The subjects were divided into three groups, which received DDT dissolved in corn oil at doses of 0, 3.5, and 35 mg. per man per day for periods up to two years. The highest dose chosen was about one-fifth of that estimated to cause transient mild sickness, based on animal experiments and on the smallest single acute dose known to produce mild illness in man. No clinical effect associated with DDT dosage was detected by laboratory testing, careful physical examination, or by the men themselves. The storage of DDT in body fat was found to be proportional to the dose. About one year was required for the men to reach equilibrium of DDT storage. Following this time no increase in DDT storage level occurred, although the men remained on the dose for an additional one-year period.

Studies such as this indicate that the storage level of DDT in fatty tissue does not increase indefinitely during continued exposure but rather reaches a plateau characteristic of any particular level of intake. Thus, if the dosage level is increased in man, the storage level would rise to the corresponding plateau for that particular level of intake. When this level or equilibrium is reached and the exposure to DDT terminated, the storage level in the fat reduces at a very slow rate. Those in the study receiving the highest dosage reached a mean level of storage of about 250 ppm. Within two years following termination of dosage the mean storage level had dropped to about 50 ppm of DDT. This illustrates that the excretion of DDT (as DDA--the water soluble acetic acid derivative of DDT) is very slow in man. Thus, any decrease in storage of DDT as the result of decrease of DDT levels in food or other environmental media would be only slowly reflected in decreased fat storage levels of the compound.

In two studies of men with occupational exposure (Ortelee, 1958; Laws et al., 1967) in manufacturing and formulating plants, clinical examinations and laboratory tests, including determination of DDA excretion, were performed on groups of 40 and 35 men, respectively, who had intensive and prolonged occupational exposure to DDT. Estimates of DDT absorption in these men were made by comparison of their urinary DDA concentrations with concentrations obtained from human volunteers on known daily oral dosages of technical DDT. In the first group the results indicated that 26 of the men had absorbed DDT at a rate equal to or higher than that associated with an oral dose of dissolved DDT at the rate of 35 mg. per man per day. The men had been exposed at this rate for 0.5 to 6.5 years (mean, 3.5 years), but showed no ill effects as judged by thorough medical examination, their own reports, or their work attendance records.

In the second group where the workers had 11 to 19 years of exposure in a plant that had produced DDT continuously and exclusively for several years, the findings from medical history, physical examination, routine clinical laboratory tests, and chest X-ray film did not reveal any detectable ill effects attributable to exposure to DDT. The overall range of storage of DDT-derived material in the men's fat was 38 to 647 ppm. Based on their storage of DDT in fat and excretion of DDA in urine it was estimated that the average daily intake of DDT by the 20 men with the highest occupational exposure was 17.5 to 18 mg. per man per day as compared to the average of 0.02 mg. per man per day I mentioned earlier that the general population received in the diet.

More extensive studies on exposed workers are now underway in contract studies being carried out by the Division of Community Studies, FDA. There are 15 of these Community Studies projects in areas of heavy pesticide usage throughout the United States. As one part of their contract, each project is asked to compile pesticide exposure and medical records of about 100 selected, high-exposure individuals. A group of individuals with minimal pesticide exposure serves as control. There are eight of these Community Study projects in the western states. One project is being carried out by the Washington State Department of Health and is located in Wenatchee.

The contractors and the Community Studies Division in examining their human subjects look not only for overt clinical illness or immediate changes, but also for the more subtle biochemical, functional, and behavioral changes, and for those that may occur on a more gradual or delayed basis.

It is evident from the information I have presented that we do have a considerable amount of data available, and more is being collected on the effect of DDT and other pesticides on human health. However, to assure that subtle effects from continued low level exposure do not occur and also to detect any possible harmful effects of new compounds, we feel that continued surveillance and research into biological effects are necessary.

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Chamblee, Georgia 30341

Original Articles

Oral Toxicity of Mirex in Adult and Suckling Rats

With Notes on the Ultrastructure of Liver Changes

Thomas B. Gaines and Renate D. Kimbrough, MD, Atlanta

The oral toxicity of mirex and its effect on reproduction was studied in rats. The one-dose oral lethal dose for 50% survival of the group (LD_{50}) in females was 365 mg/kg/day; the 90-dose oral LD_{50} was 6 mg/kg/day. The chronicity factor was 60.8. Females fed 25 ppm mirex had fewer offspring born alive, significantly fewer offspring survived to weaning, and many pups developed cataracts. Females fed 5 ppm produced normal litters. Offspring born to mothers fed 25 ppm mirex and nursed by nontreated foster mothers had a low incidence of cataracts and a normal survival rate to weaning. Gas chromatographic analysis of milk and fetuses showed excretion of mirex in the milk and passage through the placental barrier. Livers of male and female rats fed 25 ppm mirex weighed significantly more than controls. Proliferation of smooth endoplasmic reticulum and osmiophilic dense bodies were observed in these livers with the electron microscope.

MIREX (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene) is used to control ants, wireworms, and earwigs, and extensively in the southeastern United States to control fireants. However, little information is available concerning its effect on mammals.

The acute oral lethal dose for 50% survival of the group LD_{50} for mirex in Sherman strain rats was reported to be 740 mg/kg in males and 600 mg/kg in females.[1] Ware and Good[2] found that 5 ppm mirex in the diet of the BALB/c strain of Swiss mice over 120 days resulted in high mortality and low fertility and fecundity rates. Swiss mice of the CFW strain treated similarly suffered a reduction in fecundity but no increase in mortality.

Egg production was not reduced in laying hens fed 300 and 600 ppm mirex in their diet for 16 weeks but hatchability of eggs from hens fed 600 ppm and survival rate of chicks from hens fed 300 ppm[3] decreased.

This paper reports results of studies on the oral toxicity of mirex in rats, including effects on reproduction and survival of offspring.

Methods

Sherman strain rats were caged individually and weighed weekly, unless otherwise specified. The mirex was technical grade with a purity of 98%.

Basic Toxicity Studies.—To determine the one-dose oral LD_{50}, mirex was given as a 5% solution in corn oil at appropriate levels to eight groups of ten adult female rats each by stomach tube as described by Gaines.[4] Survivors were observed for a minimum of 15 days after treatment.

The 90-dose oral LD_{50} and chronicity factor described by Hayes[5] were determined in five groups of ten adult female rats, each fed 50, 100, 150, 200, and 250 ppm mirex in the diet for 90 days. The rats were distributed into groups according to a table of random numbers. Food consumption was measured daily during weeks 1, 2, 7, and 12 to determine mirex intake in milligrams per kilogram per day. After 90 days' exposure, surviving rats were fed nontreated food and observed for two weeks to allow time for death or recovery of sick animals.

The method of Litchfield and Wilcoxon[6] was used to calculate LD_{50} values.

Reproduction Studies.—Rats were bred by pair-mating for seven days. Those fed mirex were maintained on the treated diet during this period. Each day until insemination was veri-

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Read in part before the eighth annual meeting of the Society of Toxicology, Williamsburg, Va, March 10, 1969.

Reprint requests to 4770 Buford Hwy, Chamblee, Ga 30341 (Mr. Gaines).

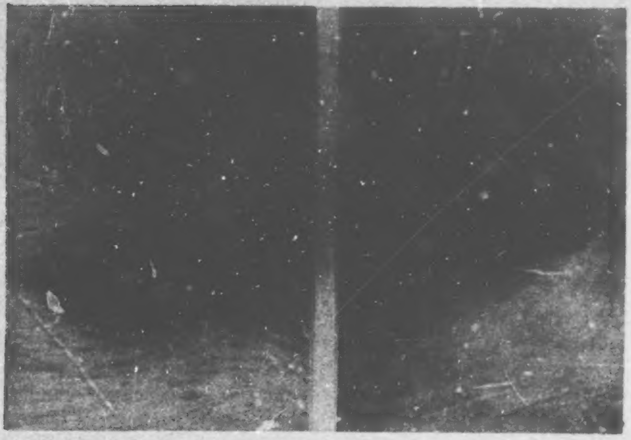


Fig 1.—**Left**, Normal weanling rat. **Right**, Weanling rat of dam exposed to 25 ppm mirex in diet for 102 days at time of breeding. Very pronounced cataract is illustrated.

fied, a vaginal smear from each female was examined microscopically for sperm. By mating mirex-treated rats with "nontreated" rats, both sexes were actually exposed to mirex during the seven days. After seven days, rats were again caged individually. Offspring were examined grossly at birth, during lactation, and at weaning for mortality and development of abnormalities. All litters were weaned when 21 days old.

Twenty female rats, about 6 weeks old, were distributed according to a table of random numbers into two groups of ten each; 20 male rats were similarly divided. One group of males and one group of females were fed 25 ppm mirex in their diet; the other two groups (controls) were fed nontreated diet. Food consumption was measured during the 1st, 6th, and 14th weeks. After 45 days' and 102 days' exposure, the rats fed mirex were mated with nontreated rats of similar strain and age from stock, and the control females were mated with the control males. The females were continued on their respective diets during gestation and lactation. Offspring resulting from the breeding at 102 days of exposure were held after weaning and fed nontreated diet until 90 to 100 days old. They were pair-mated within the respective groups, and their offspring held until weaning and checked for abnormalities.

Since 25 ppm mirex in the diet did not affect male fertility, only females were exposed to mirex in further reproduction studies. Twenty female rats, 6 to 7 weeks old, were distributed into two groups of ten each. One group was fed 5 ppm mirex in the diet and the other the nontreated diet for 102 days. Both groups were bred to nontreated males from stock of the same age and strain. The respective diets were continued during gestation and lactation.

Abnormalities in suckling rats born to treated dams may have been due primarily to excretion of mirex or its metabolites in the milk, or to the sucklings having consumed some treated food after about the age of 2 weeks. Additional studies were made to distinguish between antepartum and postpartum effects of mirex. Forty 6-week-old female rats were divided into four groups of ten rats each, according to a table of random numbers. One group was given 25 ppm mirex in the diet (treated rats); the other three groups nontreated diet. After 49 days' feeding, all females were bred to nontreated male rats of the same age and strain. During the pregnancy and postpartum period, the animals were continued on their respective diets. At birth, offspring of treated dams were transferred to foster mothers fed nontreated diet and their offspring transferred to treated dams. Offspring from the two groups that received nontreated diets (controls) were switched between the two groups, allowing us to observe the influence of the transfer on morbidity and mortality of sucklings.

Mirex in the milk of lactating dams was checked by chemical analysis of stomach contents of two 4-day-old litters of rats from dams treated 78 days and two litters of 4-day-old rats from nontreated dams. Litters were removed from mothers for six hours and then returned to nurse. One hour later, stomachs of the offspring were removed and the contents pooled by litter for analysis.

Four treated dams and four nontreated dams were killed on the 19th day of gestation; four fetuses from each dam were taken for chemical analysis. Stomach contents and fetuses were frozen until analysis for mirex by electron-capture gas chromatography.

Pathology.—Groups of 20 rats (ten males and ten females) were fed 25, 5, 1, and 0 ppm mirex in the diet for 166 days, then killed and the livers studied by light microscopy (in a preliminary study, morphological change was observed only in the liver). Tissue was fixed in buffered 10% formaldehyde solution; routine hematoxylin-eosin stains were used.

Electron microscopic studies were performed on liver tissue from five male and three female rats fed 25 ppm mirex and two males and two females fed nontreated diet (controls) for 166 days. Tissue was fixed in chilled 5% buffered glutaraldehyde for two hours, postfixed for two hours in 1% phosphate buffered osmium tetroxide, dehydrated through changes of alcohol and propylene oxide, and embedded in resin (Maraglas). Sections were cut with a glass knife. The tissue was stained with lead citrate[7] and examined with an electron microscope. The animals were not fasted prior to killing.

Results

Basic Toxicity Studies.—The one-dose and 90-dose LD_{50} values and chronicity factor for adult female rats are included in Table 1. Although the one-dose LD_{50} value of 365 mg/kg is lower than that of 600 mg/kg reported previously for adult female rats,[1] the values, as indicated by the spread of their confidence limits, are not significantly different. The chronicity factor (ratio of the one-dose LD_{50} to the 90-dose LD_{50}) of 60.8 indicates that mirex is highly cumulative. Rats fed the highest dietary level of mirex (250 ppm or 11.6 mg/kg/day) died in 16 to 60 days. Signs of poisoning were diarrhea, weakness, and hyperexcitability.

Reproduction Studies.—Results are shown in Table 2. Reproduction in male rats fed 25 ppm and in females fed 5 ppm and bred to nontreated rats was comparable with that in the controls. Females fed 25 ppm mirex (treated) for 45 days and then bred to nontreated males had significantly smaller litters than controls; the survival rate to weaning was significantly reduced ($P < 0.05$) and 33.3% of the offspring developed cataracts (Fig 1). Compared to controls, the same females bred after 102 days' exposure had fewer litters; the survival rate of offspring was significantly lower ($P < 0.05$) and 46.2% of the offspring developed cataracts. When rats developed cataracts, they were present by 21 days of age. No cataracts were observed in offspring of control rats nor in the parent stock fed mirex.

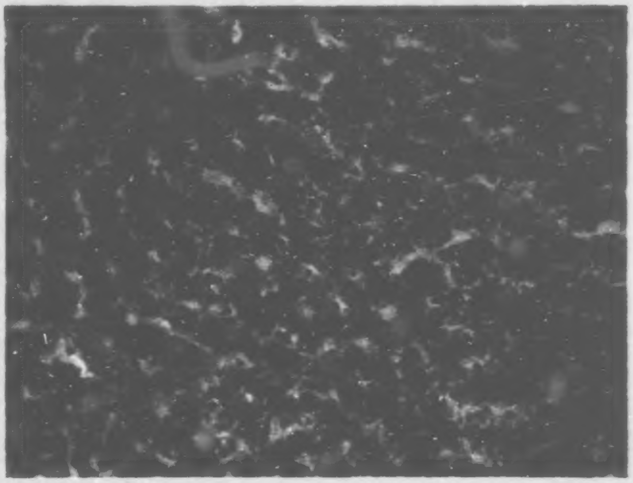
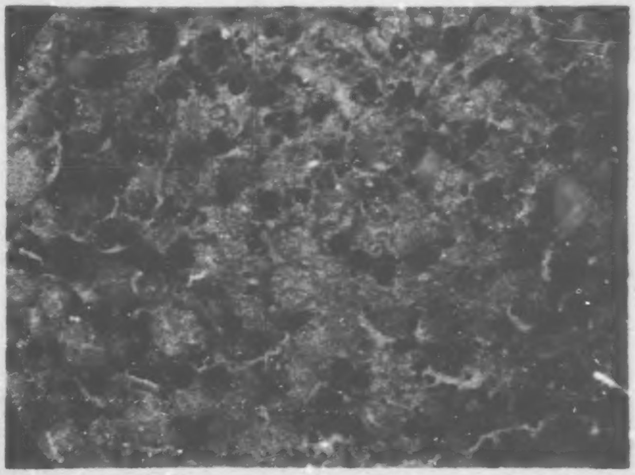


Fig 2.—Normal rat liver (hematoxylin-eosin, reduced from × 300).

Fig 3.—Liver of male rat fed 25 ppm mirex for 166 days showing enlargement of cells, bile pigment, and inclusions (arrows) within cytoplasm (hematoxylin-eosin, reduced from × 300).



Ten male and ten female offspring of females treated for 102 days were fed nontreated diet after weaning and pair-mated at 90 to 100 days of age. At the same time, ten male and ten female offspring of controls were pair-mated. In litter size and survival rate, the F_2 generation (descendants of mirex treated females) were comparable with the control F_2 generation; none developed cataracts.

Table 3 shows the antepartum effect of mirex (litters born to dams fed mirex and transferred at birth to nontreated foster mothers) and its postpartum effect (rats born to nontreated dams and transferred at birth to treated foster mothers). The survival rate to weaning of rats (controls) transferred at birth from dams fed nontreated diet to foster mothers fed nontreated diet was 95.8%; none developed cataracts. The survival rate of rats exposed to mirex in utero only was lower than that for controls (72.7% vs 95.8%), but this was not statistically significant; only 1.6% developed

Table 1.—*Oral Toxicity of Mirex to Adult Rats*

Parameter	1-Dose	90-Dose
No. of rats tested	70	50
Survival time (days)	2-12	16-74
LD_{50}*	365	6.0
19/20 confidence limits*	281-475	4.4-8.2
Lowest dose to kill*	100	6.2
LD_1*	60	1.8
Lowest dose causing clinical signs*	100	6.2
Lowest dose tested*	50	3.1†
Chronicity factor (1-dose LD_{50} ÷ 90-dose LD_{50})	60.8	

* Expressed as milligrams per kilogram for one-dose test or milligrams per kilogram per day for 90-dose test.

† Dietary concentration of 50 ppm.

Fig 4.—Normal rat liver. **SER,** Smooth endoplasmic reticulum; **RER,** rough endoplasmic reticulum; **G,** glycogen; **M,** mitochondrion; **N,** nucleus (lead citrate, × 10,600).

Table 2.—*Survival of Offspring and Incidence of Cataracts in Litters of Rats When One Parent Is Fed Mirex in the Diet*

Sex Poisoned	Dietary Level (ppm)	Dosage Level (mg/kg/Day)	Days on Diet*	No. of Matings	No. of Litters	Average No. per Litter		Survival (%)	Offspring With Cataracts (%)
						Born Alive	Weaned		
None†	0	...	...	10	9	12.0	10.7	89	0
F	25	2.8-1.8	45	10	8	8.5‡	4.5‡	53	33.3
M	25	3.1-1.3	45	10	10	11.4	10.1	89	0
None†	0	...	...	10	9	12.0	11.3	94	0
F	5	0.49-0.31	102	10	9	11.2	10.9	97	0
F	25	2.8-1.8	102	10	6	10.7	6.5‡	61	46.2
M	25	3.1-1.3	102	10	10	12.5	11.5	92	0

* At time of mating and continued on same diet during gestation and lactation.
† Control animals, male and female.
‡ Significantly less than controls ($P < 0.05$).

Table 3.—*Survival of Offspring and Incidence of Cataracts in Litters of Rats Born to Mirex-Treated Dams* and Nontreated Dams†*

Treatment		No. of Litters	Average No. per Litter		Survival (%)	Offspring With Cataracts (%)
Mothers	Foster Mothers		Born Alive	Weaned		
None‡	None‡	14	11.9	11.4	95.8	0
Mirex§	None	8	11.0	8.0	72.7	1.6
None	Mirex§	9	9.9	5.3	53.9‖	37.5

* Transferred at birth to nontreated dams.
† Transferred at birth to treated dams.
‡ Control animals.
§ Five parts per million of mirex in diet for 73 days at the time the litters were transferred.
‖ Significantly less than controls ($P < 0.05$).

Table 4.—*Relative Liver Weights and Morphological Changes in the Liver of Rats Fed Mirex (166 Days on Diet; 10 Animals per Group)*

Dietary Level	Sex	Relative Liver Weight		Remarks
		Fraction of Body Weight (%)	Difference From Controls	
0 (control)	M	2.85	...	LM* Normal liver EM† Livers of 3 rats examined; occ myelin figures and lipid vacuoles
1 ppm (0.09-0.04 mg/kg/day)	M	2.99	...	LM 2/10 rats, slightly enlarged liver cells
5 ppm (0.48-0.21 mg/kg/day)	M	2.77	...	LM 5/10 rats, slightly enlarged liver cells, smooth or vacuolated cytoplasm, occ inclusions
25 ppm (3.1-1.3 mg/kg/day)	M	4.19	$P < 0.001$	LM 10/10 rats, enlarged liver cells, multinucleation, cytoplasm smooth or vacuolated, inclusions; 5/10 rats, bile stasis EM 5 rats examined; increase in smooth endoplasmic reticulum, free ribosomes, lipid vacuoles, and myelin figures; less glycogen, osmiophilic dense bodies
0 (control)	F	3.30	...	LM Normal livers
1 ppm (0.10-0.06 mg/kg/day)	F	3.47	...	LM Normal livers
5 ppm (0.49-0.31 mg/kg/day)	F	3.66	...	LM 3 of 10 rats had enlarged liver cells and vacuolated cytoplasm
25 ppm (2.8-1.8 mg/kg/day)	F	4.24	$P < 0.025$	LM 5 of 10 rats had enlarged liver cells, multinucleation, cytoplasm smooth or vacuolated, inclusions EM Livers of 3 rats examined; increase in smooth endoplasmic reticulum and free ribosomes, myelin figures; osmiophilic dense bodies

* LM, light microscope.
† EM, electron microscope.

cataracts. When rats born to nontreated mothers were exposed postpartum only to mirex-treated mothers until weaned, their survival rate was significantly lower than that for controls (53.9% vs 95.8%, $P < 0.05$); 37.5% developed cataracts.

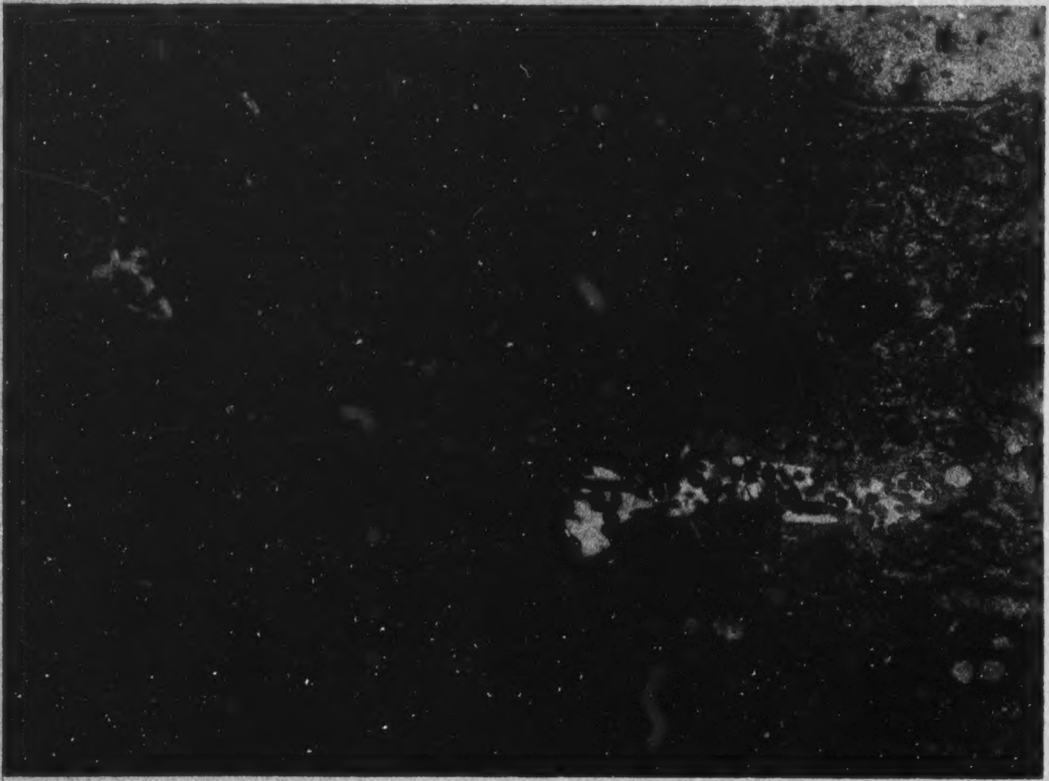
Table 3 indicates that the low survival rate and development of cataracts in offspring of treated dams (Table 2) was caused chiefly by mirex in the mother's milk. The stomach contents of two litters of 4-day-old sucklings born to mothers treated for 78 days were taken just after nursing and analyzed for mirex; the mean value was 11.3 ppm. No mirex was detected in the stomach contents of control sucklings.

Fetuses from four rats treated for 78 days were taken on the 19th day of gestation and analyzed. They contained 0.14 to 0.45 ppm of mirex (mean, 0.23 ppm); none was detected in fetuses from controls. Apparently, mirex passes through the placental barrier in rats.

Pathology.—The weights of brains, hearts, kidneys, lungs, and spleens in rats treated for 166 days did not differ significantly from those of controls. Table 4 shows relative weights of livers and a summary of morphological changes seen with the light and electron microscopes in livers of rats fed 0 (control), 1, 5, and 25 ppm mirex in the diet for 166 days.

Light microscopic changes (Fig 2 and 3) included enlargement of liver cells with either vacuolated or smooth-appearing cytoplasm containing one or more eosinophil-staining inclusions. Livers of three female and five male rats given 25 ppm mirex in

Fig 5.—Liver from male rat fed 25 ppm mirex in diet for 166 days. M, Mitochondrion; G, glycogen; SER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum; DB, vacuolated dense bodies probably representing atypical lysosomes; N, nucleus; CB, cellular border. Note increase in smooth endoplasmic reticulum, decrease in glycogen (lead citrate, × 10,600).



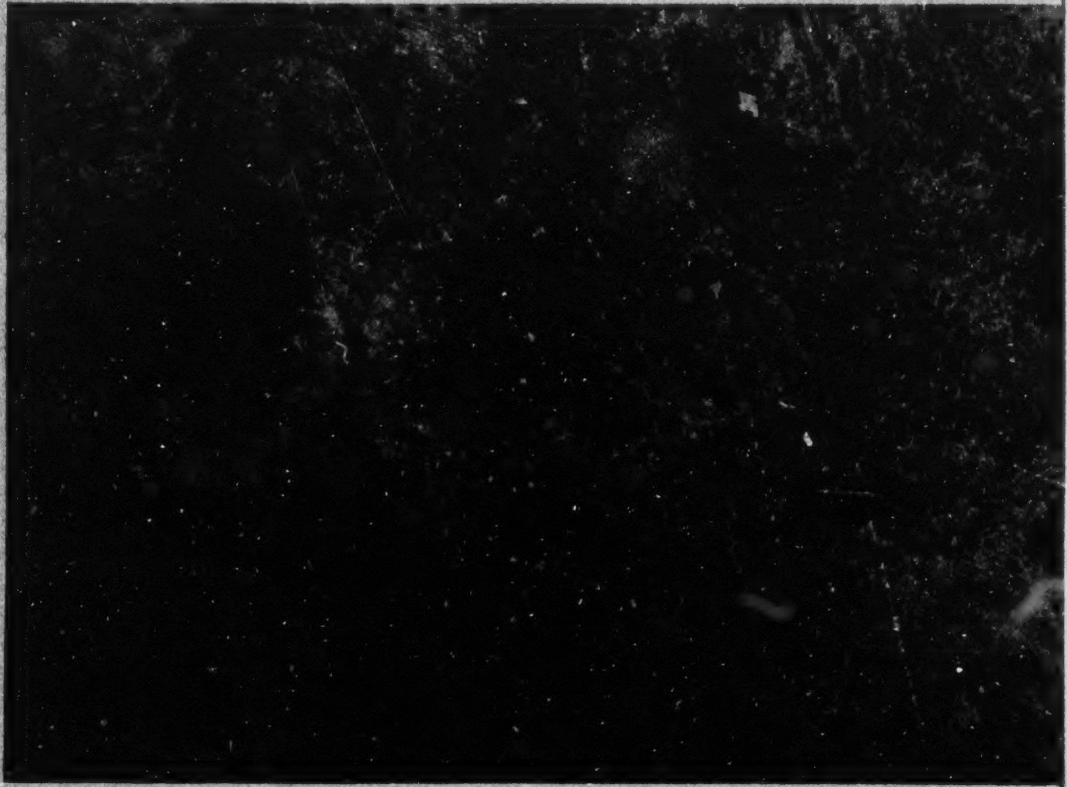
their diets, examined under the electron microscope (Fig 4 to 6), showed an increase of smooth endoplasmic reticulum. Some areas of a number of cells showed an accumulation of free ribosomes. Lipid vacuoles (also seen in control rats) increased; glycogen content in glycogen storage areas of the cells decreased. The cytoplasm of exposed rats exhibited osmiophilic electron-dense bodies, possibly large lysosomes which contained small lipid vacuoles surrounded by varying amounts of dark granular material. Small and large myelin figures were also observed. The bile canaliculi showed no apparent ultrastructural changes.

Comment

Mirex given as an acute oral dose is not very toxic. The 90-dose LD_{50} studies show that it has a very high chronicity factor of 60.8 (ratio of the one-dose LD_{50} to the 90-dose LD_{50}). In contrast, the chronicity factor in female rats is only 5.4 for DDT and 12.8 for dieldrin. These findings indicate that mirex is highly cumulative in effect; like many other chlorinated hydrocarbons, it is probably metabolized or excreted very slowly.

The number of offspring is reduced in treated dams. It is not clear whether this is due to death of the fetus in utero or to a lower fecundity rate. Cataracts develop predominantly in pups in the suckling period. However, when offspring are transferred from mirex-fed mothers to nontreated foster mothers at birth, the incidence of cataracts is very low. Chemical analyses of fetuses of dams exposed prior to birth and of the milk of exposed dams indicates that mirex passes

Fig 6.—Liver from male rat fed 25 ppm mirex in diet for 166 days. M, Mitochondrion; SER, smooth endoplasmic reticulum; G, glycogen; MF, myelin figures (lead citrate stain, × 42,600).



through the placental barrier and is excreted in the milk. Thus, the offspring are exposed to mirex both in utero and after birth, but it is the presence of mirex in the milk after birth that leads to the high incidence of cataracts. If dams are fed 5 ppm mirex for 102 days at the time of breeding and continued on this diet until their offspring are weaned, the pups do not develop cataracts. The number of animals born alive and the survival rate to weaning are also normal.

The effect of mirex on livers is like that of other chlorinated hydrocarbons such as dieldrin and DDT; liver weight increases. Individual hepatocytes are larger than those of controls. Mirex also causes bile stasis, which has not been observed with DDT.

Electron microscopic studies show increased smooth endoplasmic reticulum, characteristic of other chlorinated hydrocarbons. The dense bodies (Fig 5), probably atypical lysosomes, have not been observed in livers of rats fed DDT.[8] According to Ortega,[8] accumulations of lipid surrounded by several concentric layers of membranes are observed in the livers of rats given high doses of DDT but not in mirex-treated rats (also according to unpublished data of one of us [R.D.K.]).

Goldfischer et al[9] observed a numerical increase and more widespread cytoplasmic distribution of lysosomes in livers of rats with ligated bile ducts and rats treated with icterogenin and 17α-ethyl-19-nortestosterone. They found that these lysosomes or dense bodies had vacuoles which did not contain electron-dense material. Similar bodies were observed in human obstructive jaundice.[10]

Electron microscopically, glycogen was less apparent in liver cells of mirex-treated animals than in controls killed at the same time; it is also less apparent in livers of rats treated with phenobarbital sodium[11] but chemical analysis of their liver tissue for glycogen shows no decrease. Glycogen content was not determined chemically in our treated rats; possibly it is not reduced but only becomes invisible with the electron microscope.

Livers of male rats are slightly more sensitive to mirex than those of females. At 1 ppm in the diet (Table 4) all livers of ten female rats were normal microscopically but two of ten livers of male rats still showed slightly enlarged liver cells.

This study demonstrates that mirex is highly cumulative and causes cataracts in offspring of dams fed mirex. Further studies should include its metabolism in animals and its breakdown in soil. Whether the effect on the liver is an adaptive change or should be considered as liver damage is not known at present in terms of an increase in smooth endoplasmic reticulum, formation of atypical lysosomes, and liver enlargement. Bile stasis, on the other hand, has always been considered a sign of liver dysfunction.

Ralph E. Linder and Richard L. Moore assisted with the animal tests. Estelle C. Gray assisted with the statistical analysis. Ralph W. Jennings, Virlyn W. Burse, and Helen T. Mann did the chemical analysis.

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Hazards Associated With Unintentional Food Additives

PUBLICATION

111

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ALL OF THE UNINTENTIONAL FOOD ADDITIVES are poisonous in some degree. This is emphasized by the fact that cases of human poisoning are known in connection with most of them that have been used in significant tonnage (Hayes, 1960). The list of such compounds applied to crops includes inorganic insecticides such as cryolite,[1] lead arsenate, and Paris green, botanical insecticides such as nicotine, pyrethrum, and rotenone; chlorinated hydrocarbon insecticides such as aldrin, BHC, chlordane, DDT, dieldrin, endrin, TDE, and toxaphene; organic phosphorus insecticides such as DDVP, demeton, Diazinon,[2] Dipterex, malathion, methyl parathion, mipafox, parathion, Phosdrin, schradan, TEPP, phorate, and Trithion; dinitro compounds used as either insecticides or plant growth regulators; hormone-type growth regulators; fungicides and seed dressings such as hexachlorobenzene and ziram; several organic mercury compounds; and growth promoters such as antibiotics. Cases of poisoning are also known in connection with pesticides that may contaminate food although they are not intentionally used on food crops. Such cases have involved the rodenticides such as ANTU, arsenic trioxide, barium carbonate, thallium, sodium monofluoroacetate, and strychnine sulfate; fumigants such as hydrogen

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[1] Terminology for insecticides is in accordance with the Committee on Insecticide Terminology of the Entomological Society of America as indicated by Smith (1959a, 1959b, and 1961).

[2] Use of trade names is for identification purposes only and does not constitute endorsement by the U. S. Public Health Service.

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8

cyanide, dibromoethane, and methyl bromide; household insecticides such as Lethane, naphthalene, and sodium fluoride; and defoliating compounds such as sodium chlorate.

Partial contents

Contamination of Foods

Food may become contaminated in many ways. Poisoning has occurred in this country when leafy vegetables were sprayed with toxaphene entirely contrary to directions and then harvested within a day or so. The residue was 3315 ppm., and 3126 ppm still remained after washing (McGee *et al.*, 1952). If toxaphene is used at all on such crops, it is supposed to be used on seedlings only so that no residue remains at harvest. The same sort of thing occurred with nicotine. Even two weeks after spraying the residue ranged from 69 to 123 ppm as compared with a permitted level of 2 ppm (Lemmon, 1956). The intentional addition of lindane to sugar by a child resulted in severe illness of four people. Poisoning by pesticides has been a much more serious problem in some other countries than in the United States. Thousands of people were made chronically sick and some were killed in Turkey when they ate grain intentionally treated with hexachlorobenzene (Schmid, 1960). In a somewhat similar way, prepared bait containing the rodenticide warfarin was used as food by a family in Korea. Each of the 14 persons in the family became sick, and two died (Lange and Terveer, 1954).

The most common and serious cause of outbreaks of nonoccupational poisoning has been the accidental contamination of grain or other staple food during shipment. Of the new compounds, parathion has been involved most frequently. An episode in the Kerala and Madras States in India followed the contamination of grain during ocean shipment by leakage of parathion from polyethylene carboys. The grain was distributed widely before the danger was detected, so that deaths continued to occur for about a month (Anonymous, 1958). At least 102 persons were killed (Karunakaran, 1958). There have been some similar occurrences in other countries. Although no such tragedy has occurred in this country, an outbreak of endrin poisoning in Wales reminds us that it can happen here. Fifty-nine persons were poisoned and 30 of them had fits. The trouble was traced to bread made from sacked flour that had been shipped in a freight car previously used to transport endrin. Much of the contamination probably came from the flour sacks, which were used to cover the loaves as they rose (Davies and Lewis, 1956).

Storage of DDT

P34

At least one pesticide, DDT, is stored in essentially everybody in this country. This phenomenon, because it involves the whole population, probably deserves as much attention as the relatively rare instances in which pesticides produce recognized illness.

A trace of DDT has been found in every whole meal analyzed, although some individual foods contained no detectable insecticide. The average daily intake of DDT is about 0.2 mg/man/day (Walker et al., 1954; Hayes et al., 1956). The storage of DDT in the fat of people in the general population is shown in Table 10.1. It is almost certainly dietary in origin. Most of the DDT in our food is in meat and related products (Walker et al., 1954). However, Eskimos from isolated Alaskan villages where the native food contained no detectable DDT stored even less DDT than meat abstainers in the 48 contiguous states (Durham et al., 1961). Thus, it is the DDT content of the food rather than the amount of animal fat and protein that is important in determining the degree of storage.

People whose work brings them in contact with DDT store more of the compound than people in the general population (Hayes et al., 1958). The amount depends on the kind of work (see Table 10.1). The fat of one healthy formulator contained a concentration of 648 ppm (Hayes et al., 1956).

Table 10.1. Concentration (ppm) of DDT and DDE in Fat of People*

Exposure Group	DDT	DDE
1. Died before DDT	0	0
2. Abstain from meat	2.3	3.2
3. General population	4.9	6.1
4. Environmental exposure	6.0	8.6
5. Agricultural applicators	17.1	22.3

*Adapted from Hayes, et al., 1958.

The relation between oral dosage of DDT and its storage in fat has been studied in volunteers (Hayes et al., 1956). An extension of this work permitted Ortelee (1958) to relate oral intake of DDT and the urinary excretion of DDA. He was able to show (Figure 10.1) that more than half of the workers in certain formulating plants excreted DDA at a rate equal to or greater than that in men taking a repeated oral dosage of 35 mg/man/day. The men had been employed at this kind of work for as long as 6.5 years.

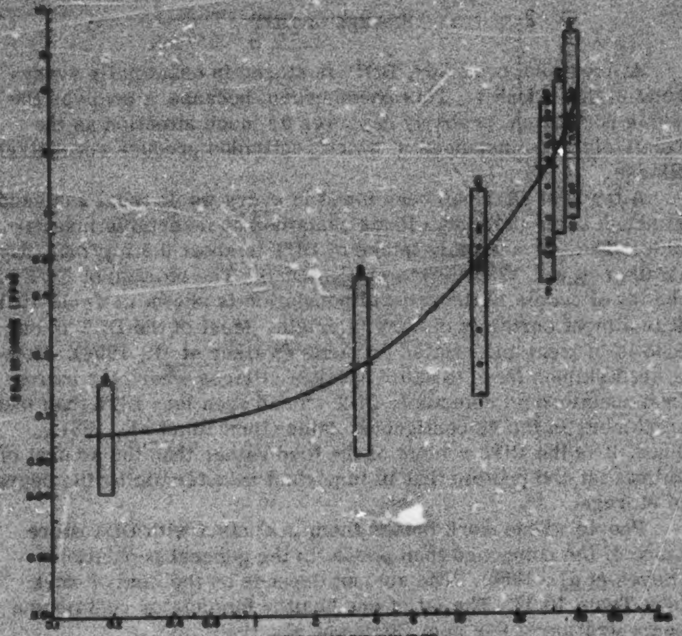

Fig. 10.1. Concentration of DDA in the urine of men with known daily oral intake of DDT (Bars A, B, and E) and of men with different degrees of industrial exposure to the compound (Bars C, D, and F). Each bar indicates the range and mean for the group. In Bars C, D, and F, corresponding to Grades 1, 2, and 3 respectively of industrial exposure, the individual values are shown. (From Ortelee, 1958, reprinted with permission from the A.M.A. Archives of Industrial Health.)

The fact that men can tolerate for years a dosage of DDT approximately 200 times that of the general population is reassuring. However, it seems best to repeat the observations periodically for at least one generation in order to detect any long-term effects that may occur.

13

Mortality Caused by Pesticides

The annual mortality in the United States from all causes is approximately 935 per 100,000, varying only slightly from year to

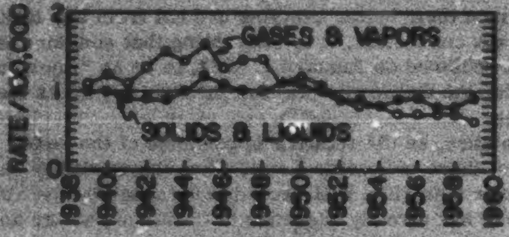

Fig. 10.2. Accidental deaths in the United States caused by chemicals expressed as rate per 100,000 population. (Based on U. S. Public Health Serv. Vital Statistics. U. S., 1936-1959 [1940-1960].

year. This rate may be compared to the rates of about 1.0 for accidental acute poisoning by gases and vapors and by solids and liquids, respectively. The exact rates over a period of several years are shown in Figure 10.2. It may be seen that there has been no dramatic change in the last two decades. The current overall rate for acute poisoning is only about one-fourth the rate at the turn of the century. People have learned to use poisons, and chemicals generally, with greater care. However, there is much room for improvement.

Each year pesticides contribute approximately 10 percent of the deaths caused by solid and liquid materials (Conley, 1958; Hayes, 1960). The deaths caused by fumigants are very few. Thus, pesticides are associated with an annual mortality rate of about 0.1 per 100,000 population in this country.

More than half of the deaths caused by pesticides are in children. For example in 1956, 62 percent of these deaths were in children less than 10 years old and 51 percent were in children less than 4 years old (Hayes, 1960). It may be more unexpected that, during 1956, the last year for which complete figures are available, 68 percent of the deaths caused by pesticides were caused by compounds introduced before DDT.

It seems that the greatest opportunity for improvement involves the older poisons and the unnecessary exposure of children. There can be no doubt that the careful labeling that has been required for all pesticides since 1947 (U.S. Laws, 1947) has been a powerful factor in educating the public in the use of these compounds. The newer pesticides have been manufactured in greater tonnage than the older ones for several years, and the newer compounds are just as toxic on the average as the older ones. It follows that the good record of the newer compounds results from their better handling and not from any lack of hazard. In this connection, it is interesting that sodium arsenite was first declared on January 1, 1962, to be an "injurious material" under the California law that directly regulates the use of pesticides.

To be sure, as the relative importance of the newer compounds increases, there will come a time when the mortality caused by these newer materials will exceed that caused by the older poisons. This has already happened in California so far as the morbidity of occupationally exposed persons is concerned. Seventy-five percent of the systemic cases resulting from occupational exposure were associated with organic phosphorus insecticides (Kleinman et al., 1960). For several years, use of the more toxic organic phosphorus compounds in California has required a special permit (California Law, 1953). Now their use will not be permitted without medical supervision (California Law, 1961).

How Others See Us

Since we not only have sufficient food but even have surpluses, we can afford to be conservative about any contamination of food by chemical residues. The same is not true in all parts of the world. Another less obvious difference is that we can afford to carry out a great deal of systematic toxicological study. But the necessity to focus technically trained talent on real problems is not confined to the developing countries; it extends to some of the most technically advanced nations (Barnes, 1961).

Problems

Real problems with unintentional food additives include our lack of adequate data on storage of pesticides in the body and our incomplete understanding of the dynamics of storage in general. A problem that potentially involves pesticides, although it affects drugs more, is the lack of an adequate test for proving or disproving a relationship between a specific chemical and blood dyscrasia in individual cases (Hayes, 1961). Finally, the possibility must be kept in mind that the most important hazards of foods may concern what is conventionally called nutrition. If sodium chloride contributes to hypertension as it does in rats (Meneely et al., 1957) and if saturated fats contribute to arteriosclerosis (Bronte-Stewart, 1961), are these not toxic effects?

* * *

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TOXICOLOGY AND APPLIED PHARMACOLOGY 20, 396–403 (1971)

Teratology Studies with 2,4,5-Trichlorophenoxyacetic Acid and 2,3,7,8-Tetrachlorodibenzo-*P*-dioxin

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Received February 22, 1971

Teratology Studies with 2,4,5-Trichlorophenoxyacetic Acid and 2,3,7,8-Tetrachlorodibenzo-*P*-dioxin: COURTNEY, K. D., and MOORE, J. A. (1971). *Toxicol. Appl. Pharmacol.* 20, 396–403. The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were teratogenic in the CD-1, DBA/2J, and C57B1/6J strains of mice. Both compounds produced cleft palates and kidney malformations. When these compounds were administered in combination, the activity was not potentiated at the doses employed. In the CD rat, 2,4,5-T was neither teratogenic nor fetotoxic. However, TCDD produced kidney anomalies. Prenatal administration of 2,4,5-T did not affect the postnatal growth and development of the CD rat.

The herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is one of the chlorinated phenoxyacid class with extensive utilization as a defoliant (Audus, 1964). Pharmacologic and toxicologic reports considered this compound to be only moderately toxic in animals (Drill and Hiratzka, 1953; Rowe and Hymas, 1954). A recent report found 2,4,5-T to be teratogenic in mice and rats (Courtney *et al.*, 1970), but it was later found that the sample of 2,4,5-T used contained 30 ppm of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a by-product formed during the commercial manufacturing process. TCDD and other closely related compounds are considered extremely toxic and have been implicated in outbreaks of chloracne in chemical workers (Schulz, 1968), lethal hepatonecrosis in rabbits (Schulz, 1968), chick edema factor (Higginbotham *et al.*, 1968), and a disease in animals fed toxic fat (Allen and Carstens, 1967). Since it was not known whether the teratogenic results previously reported were due to 2,4,5-T, TCDD or the combination, a study was initiated in mice and rats to determine the teratogenic agent.

MATERIALS AND METHODS

Two different samples of 2,4,5-T and one sample of TCDD were used. All samples were supplied by Dow Chemical Company, Midland, Michigan. The samples of 2,4,5-T designated by the authors as technical (2,4,5-T/tech) and analytical (2,4,5-T/anal) grade were analyzed by the Dow Chemical Company; the results of their analyses are presented in Table 1. The analysis of TCDD found it to be of greater than 99% purity.

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* Present address: Perrine Primate Laboratory, EPA, p.o. Box 490, Perrine, FL 33157.

TABLE 1

CHEMICAL ANALYSIS OF ANALYTICAL AND TECHNICAL GRADE SAMPLES OF 2,4,5-T. ANALYSES SUPPLIED BY DOW CHEMICAL COMPANY

Impurities	Quantity of impurities	
	Technical[a]	Analytical
TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin)	0.5 ppm	<0.05 ppm
2,5-Dichlorophenoxyacetic acid	0.42%	<0.1%
2,4-Dichlorophenoxyacetic acid	0.05%	<0.1%
2,6-Dichlorophenoxyacetic acid	<0.02%	0
2,3,6-Trichlorophenoxyacetic acid	0.55%	<0.1%
2,4,6-Trichlorophenoxyacetic acid	<0.1%	0
Bis(2,4,5-trichlorophenoxy) acetic acid	0.4%	0
3 Isomers of dichloromethoxyphenoxyacetic acid	2.9%	0.2%
2,4,5-Trichlorophenol	<0.1%	0

[a] Dow Lot No. 120449.

Three strains of mice and 1 strain of rat were used. The random-bred mouse, CD-1 strain, was considered the animal of choice for these studies since it would allow for the expression of results against a heterogeneous genetic background. To assess results against a homozygous genetic background, two inbred strains of mice, DBA/2J and C57B1/6J, were also used. Rats of the CD strain were used. Both the CD-1 mice and CD rats were procured from Charles River Laboratories, Wilmington, Massachusetts. The mice of the two inbred strains, DBA/2J and C57B1/6J were procured from the Jackson Laboratories, Bar Harbor, Maine and subsequently mated at the Institute. Detection of a vaginal plug in mice and presence of sperm in vaginal smears from rats indicated day 0 of pregnancy. All animals were primiparous females, housed individually, and allowed free access to food and water.

2,4,5-T and TCDD were administered sc as solutions in 100% $(CH_3)_2SO$ (dimethyl sulfoxide) in volumes of 100 μl per mouse for each injection. This procedure was selected since a previous study (Courtney, *et al.*, 1970) showed that the po or sc route yielded equivalent incidences and types of anomalies. 2,4,5-T was administered to rats by gastric intubation as a suspension in 15% sucrose solution; volumes of 200 μl per rat were used for each administration. TCDD was administered sc to rats in $(CH_3)_2SO$ in volumes of 100 μl per injection. In all animals, the administration of compounds commenced on day 6 of gestation and continued on a daily basis through day 15 of gestation.

CD-1 strain mice were killed on day 17 of gestation, C57B1/6J and DBA/2J strains of mice on day 18 of gestation, and CD strain rats on day 20 of gestation. All animals were killed by decapitation after which both mother and fetuses were examined grossly. Fetuses were fixed in Bouin solution until necropsied.

The following conventions were observed in compiling data. If a fetus was either dead or resorbed, it was regarded as a dead fetus. Only live fetuses were examined for physical abnormalities. A fetus was classified abnormal if it was alive and had at least one type of anomaly (regardless of type). Similarly, a litter was classified as abnormal if it contained one or more abnormal fetuses. A fetus was said to have abnormal kidneys if at least one of its kidneys was affected. In calculating the ratios of liver to body weight in the mother, maternal body weight was defined as the difference between the weight of the mother on the day it was killed and the gravid uterus weight. Maternal weight gain was defined as the difference in the adjusted maternal weight on the day it was killed and its weight on day 6 of pregnancy.

For statistical analysis, the litter was considered the experimental unit. Five litters or less were considered an insufficient sample size for statistical analysis. Averages were calculated for each litter, then across litters. The Mann Whitney U and chi square tests were used.

RESULTS AND COMMENTS

The 3 experiments with the CD-1 mouse are presented separately so that the experimental animals could be compared to their respective controls. This was preferred due to slight variations observed in the randombred mouse.

Table 2 presents the toxicologic evaluation of 2,4,5-T and TCDD in the pregnant mouse and rat. It had been reported earlier that $(CH_3)_2SO$ was neither toxic nor teratogenic under the conditions of this experimental design (Courtney *et al.*, 1970). The current data confirm this observation. The following results are shown in Table 2.

Fetal mortality. 2,4,5-T/tech was fetocidal in the CD-1 mouse at a dose of 150 mg/kg which may be close to a maternal toxic dose. At all other doses studied, 2,4,5-T/tech or 2,4,5-T/anal did not affect fetal viability in the mouse. In the rat, there was a significant increase in fetal mortality at a dose of 80 mg/kg. Since this dose was a maternal LD40, the increase in fetal mortality may be related to maternal toxicity. TCDD did not increase fetal mortality in the mouse or rat at the doses employed.

Fetal weight. In all mouse strains, both samples of 2,4,5-T, at 100 mg/kg and greater, significantly reduced fetal weight. The lowest dose of 2,4,5-T, in experiment 1 with the CD-1 mouse, did not affect fetal weight. An apparent dose-related response is seen in this experiment and experiment 3. The reduction in fetal weight is probably a toxic action on the fetus. The administration of 2,4,5-T/tech to rats did not affect fetal weight. TCDD did not significantly affect fetal weight of either mice or rats.

Maternal weight gain. Maternal toxicity was evaluated by the maternal weight gain during gestation. In the studies with the C57B1/6J mice, 2,4,5-T/tech produced a significant reduction in maternal weight gain. In all other mouse studies, neither 2,4,5-T/technical, nor 2,4,5-T/analytical, nor TCDD significantly affected maternal weight gain. In the rat, 2,4,5-T/tech caused a reduction in maternal weight gain at the 2 highest dose levels while TCDD was without significant effect. In general, a reduction in maternal weight gain suggests some maternal toxicity.

Maternal liver to body weight ratio. Both samples of 2,4,5-T caused a significant increase in the ratio of maternal liver to body weight in all 3 strains of mice. In the CD-1 mouse, TCDD did not affect this parameter, but did produce a significant increase in this ratio in the inbred strains of mice. In the rat studies, neither 2,4,5-T nor TCDD affected this ratio. The significance of liver enlargement due to pesticides has been extensively discussed by Golberg (1966). It is attributed in many cases to a hyperplasia and concomitant increase in the metabolism of pesticides, stimulated or induced by the pesticide itself. In the rat, there was no increase in the ratio of liver weight to body weight, and the rat does not readily metabolize 2,4,5-T unless it is induced (Courtney, 1970). In contrast, with 2,4,5-T stimulation there was liver enlargement in the mouse,

TABLE 2

Toxicologic Evaluation of 2,4,5-T and TCDD[a]

Species	Compound	Dose d	No of litters	Average % fetal mortality/litter	Average fetal weight (g)		Maternal weight gain (g)		Maternal liver/body weight × 100	
					$\overline{X}$	SD	$\overline{X}$	SD	$\overline{X}$	SD
CD-1 mouse, random bred										
Expt. 1	DMSO c	a	12	6.6	1.35	0.09	2.25	2.00	7.73	0.37
	2,4,5-T/tech	50	7	6.6	1.26	0.07	2.87	1.84	8.22	0.72
	2,4,5-T/tech	100	3	7.5	1.00	0.07	4.76	0.89	9.35	0.44
	2,4,5-T/tech	150	4	51.7	0.91	0.14	4.14	2.45	10.23	0.67
Expt. 2	DMSO	a	9	8.8	1.02	0.20	2.92	1.54	8.06	0.50
	2,4,5-T/anal	100	9	9.6	0.73*	0.16	2.97	1.76	9.51*	0.96
	TCDD	1	9	13.6	1.07	0.24	2.44	1.93	7.62	0.95
	TCDD	3	10	12.4	0.96	0.24	2.80	1.85	8.54	0.41
Expt. 3	DMSO	a	8	8.4	1.09	0.20	3.44	1.86	8.59	1.08
	2,4,5-T/tech	100	10	10.7	0.85*	0.13	4.46	2.22	10.18*	0.46
	2,4,5-T/anal	100	10	11.6	0.86*	0.17	3.17	2.85	10.53*	1.20
	2,4,5-T/anal	125	9	12.9	0.71*	0.25	3.75	4.16	10.99*	0.70
	2,4,5-T/anal + TCDD	100 + 1	10	11.8	0.78*	0.11	3.75	1.85	10.69*	0.55
DBA/2 mouse, inbred	DMSO	a	24	26.1	0.85	0.11	3.82	1.68	8.65	0.64
	2,4,5-T/tech	100	11	27.0	0.67*	0.10	2.37	2.15	9.72*	0.78
	TCDD	3	9	27.0	0.89	0.13	3.97	1.89	9.15*	0.38
C57B1/6 mouse, inbred	DMSO	a	24	10.8	0.99	0.09	3.35	1.48	8.00	0.60
	2,4,5-T/tech	100	15	15.9	0.75*	0.12	1.67*	1.68	10.39*	1.00
	TCDD	3	7	5.3	1.05	0.16	3.25	1.27	9.31*	0.30
CD rat, random bred										
Group 1	Sucrose	a	29	3.4	2.48	0.25	65.56	11.56	5.17	0.36
	2,4,5-T/tech	10	10	1.8	2.40	0.38	61.13	11.44	5.23	0.39
	2,4,5-T/tech	21.5	8	1.4	2.54	0.20	63.83	9.19	5.04	0.25
	2,4,5-T/tech	46.4	7	3.8	2.20	0.42	43.21*	19.80	5.29	0.48
	2,4,5-T/tech	80.0 b	4	52.1	2.30	0.20	27.25	25.17	5.95*	0.31
Group 2	DMSO	a	9	0.0	2.44	0.05	69.70	13.15	5.35	0.39
	TCDD	0.5	6	3.0	2.41	0.17	57.96	10.12	5.65	0.45

[a] Abbreviations: a = 100 μl/animal; b = maternal LD40; * = $p = <0.05$; c = dimethyl sulfoxide; d = TCDD expressed as μg/kg; 2,4,5-T expressed as mg/kg.

and the mouse does show an increase in microsomal drug metabolism (unpublished data).

Cleft palate. The teratogenic evaluation of 2,4,5-T and TCDD is presented in Table 3. Both samples of 2,4,5-T produced cleft palate in all 3 mouse strains. A dose-related response is suggestive in the CD-1 mouse with 2,4,5-T/tech in experiment 1 and is apparent in experiment 3 with 2,4,5-T/anal. Also, in experiment 3, both samples of 2,4,5-T at 100 mg/kg were equivalent in response since the number of litters affected and the incidence of cleft palate in these litters were essentially the same.

TCDD also produced cleft palate in all 3 strains of mice. In experiment 2, with the randombred mouse, TCDD was studied at 2 dose levels. At the lower dose level, 1 μg/kg, only 1 litter had fetuses with cleft palate, while at the 3 μg/kg dose, 3 out of 10 litters had fetuses with cleft palate. Also, in experiment 3, TCDD was combined with 2,4,5-T/anal to determine whether the response of 1 compound was affected by the presence of the other. Cleft palates were produced by the combination but there appeared to be no potentiation of the teratogenic effect.

Neither 2,4,5-T nor TCDD produced cleft palate in the rat fetuses. An additional study was undertaken to evaluate the possibility that 2,4,5-T could cause cleft palate in the rat. Nine rats received 2,4,5-T/tech, 150 mg/kg, sc on days 13 and 14 of gestation, the time of palate closure. This is the maximum dose which could be tolerated for 2 days and not cause maternal mortality. No cleft palates were observed in these fetuses; however, there was an increase in fetal mortality (14%) which was statistically significant compared to the control value (0%).

Kidney anomalies. The other anomaly to be considered in these studies involves the kidney. All kidneys were sliced open and examined grossly. The anomalies observed could be described as unilocular cystinephrotic kidney as well as hydronephrosis of all gradations. Histopathologic studies are under way to clearly define the renal malformations. Until further definition is accomplished, all types of kidney malformations are simply tallied as kidney anomalies.

As can be seen from the 3 experiments with the CD-1 mouse, the incidence of renal anomalies in the control animals varied. In the first experiment no kidney malformations were observed, whereas in the second and third experiments there was a low incidence which may be considered normal for this strain. The 2 inbred strains also had a low background incidence of kidney anomalies.

In experiment 1 with the CD-1 mouse, 2,4,5-T/tech did not affect kidney development. In the fetuses from mice treated with 2,4,5-T/anal, 100 mg/kg (experiments 2 and 3), there was an increase in the incidence of litters affected and also in the average number of fetuses in these litters displaying kidney malformations. Administration of 2,4,5-T/tech did not produce as great a response. In the studies with the inbred strains of mice, 2,4,5-T/tech did not increase in the incidence of kidney anomalies relative to the control values.

Treatment with TCDD produced a marked increase in the incidence of kidney anomalies in all mice strains, and a dose-related response is evident in experiment 2 with the CD-1 mouse. There was no potentiation of the response when 2,4,5-T/anal was administered in combination with TCDD. The mouse studies showed that the C57B1/6J mouse was the most sensitive strain to TCDD-induced effects in that almost 100% of the fetuses displayed kidney anomalies.

TABLE 3

INCIDENCE OF CLEFT PALATE AND KIDNEY ANOMALIES IN 2,4,5-T AND TCDD-TREATED MICE AND RATS[a]

					Cleft palates		Kidney anomalies	
Species	Compound	Dose d	No. live litters	Av. No. live fetus/litter	No. litters affected	Av. No. cleft palates in affected litters	No. litters affected	Av. No. affected fetuses in affected litters
Mouse								
CD-1, random bred								
Expt. 1	DMSO c	a	12	11.8	0	0	0	0
	2,4,5-T/tech	50	7	12.0	0	0	0	0
	2,4,5-T/tech	100	3	11.7	1	3.0	0	0
	2,4,5-T/tech	150	3	9.0	3	5.3	0	0
Expt. 2	DMSO	a	9	11.4	0	0	3	1.0
	2,4,5-T/anal	100	9	11.8	8	4.4	7	1.7
	TCDD	1	9	9.4	1	2.0	5	4.6
	TCDD	3	10	10.4	3	1.0	10	6.5
Expt. 3	DMSO	a	8	11.3	0	0	5	2.0
	2,4,5-T/tech	100	10	10.4	4	2.0	8	2.4
	2,4,5-T/anal	100	10	9.8	4	2.0	10	4.2
	2,4,5-T/anal	125	9	9.7	7	5.4	6	4.3
	2,4,5-T/anal + TCDD	100 + 1	10	10.9	4	3.3	8	5.3
DBA/2J, inbred	DMSO	a	23	5.1	0	0	3	1.0
	2,4,5-T/tech	100	11	3.7	3	1.0	1	1.0
	TCDD	3	9	6.1	2	1.0	8	1.8
C57B1/6J, inbred	DMSO	a	23	7.2	0	0	2	1.0
	2,4,5-T/tech	100	15	6.7	6	1.2	0	0
	TCDD	3	7	8.3	5	2.6	7	8.0
Rat								
CD random bred	Sucrose		29	9.3	0	0	0	0
	2,4,5-T/tech	10	10	10.2	0	0	2	1.0
	2,4,5-T/tech	21.5	8	10.6	0	0	3	1.3
	2,4,5-T/tech	46.4	7	8.9	0	0	1	2.0
	2,4,5-T/tech	80 b	2	12.5	0	0	1	4.0
	DMSO	a	9	11.6	0	0	0	0
	TCDD	0.5	6	8.0	0	0	4	1.8

[a] Key to letters: a = 100 μl/animal; b = maternal LD40; c = dimethyl sulfoxide; d = TCDD expressed as μg/kg; 2,4,5-T expressed as mg/kg.

In the rat, 2,4,5-T/tech produced a minimal response in kidney malformations while TCDD produced a slightly greater response; therefore, additional studies were undertaken in the rat to evaluate the effect of TCDD on kidney development. TCDD was administered on days 9 and 10 (6 rats) or days 13 and 14 (8 rats) of gestation at a dose of 2 μg/kg, in $(CH_3)_2SO$ sc. In the control rats, there were no fetuses with kidney anomalies as compared to an 11% incidence from treatment with TCDD on days 9 and 10 and a 34% incidence from treatment on days 13 and 14. Thus, it appears that TCDD can affect kidney development in the rat fetus. In these studies, no cleft palates were observed in the control or experimental fetuses.

Some sources of species and strain differences in teratology have been discussed by Baker *et al.* (1970) and Smithberg (1967); however, until the mechanism of action of 2,4,5-T or any other teratogen is more fully understood, it is difficult to ascribe any one cause or causes to the difference in species response. Currently, the fetal distribution of 2,4,5-T is being studied in the mouse and rat to see whether this might be a reason for the difference in response of these 2 species.

Other anomalies. A few additional types of anomalies were observed in the fetal mice and rats. In general, the type and incidence of these anomalies were similar in the control and experimental animals.

Also, in this study, 2 very mild hemorrhagic gastrointestinal tracts were seen in fetuses from 2,4,5-T treated rats and 6 hemorrhagic gastrointestinal tracts in fetuses from TCDD treated rats. Hemorrhagic gastrointestinal tracts are probably a manifestation of toxicity as opposed to a malformation. In this case, the toxicity is most likely directly on the fetus as opposed to maternal toxicity being reflected in the fetus.

Postnatal study. In order to confirm that 2,4,5-T was not teratogenic in the rat, a postnatal study was undertaken. Eight pregnant rats were administered 2,4,5-T/anal, 50 mg/kg, po, in sucrose from day 6 through 15 of gestation. Seven control rats received 0.2 ml sucrose. All rats were allowed to litter. The neonates were examined and weighed on day 1 and twice weekly thereafter until sacrificed on day 21. Litter size and weight of the 2,4,5-T/anal treated litters were comparable to the controls at all times. Mortality was less than 1% in both groups. The weight gain for the 3 wk period was similar for both groups. Eye opening occurred on days 13 and 14 for all neonates, both treated and controls. Thus, under the conditions of these experiments, 2,4,5-T is not teratogenic in this strain of rat and prenatal administration does not impair postnatal growth and development.

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Toxicological Assessment of Hexachlorobiphenyl Isomers and 2,3,7,8-Tetrachlorodibenzofuran in Chicks

II. Effects on Drug Metabolism and Porphyrin Accumulation[1]

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Toxicological Assessment of Hexachlorobiphenyl Isomers and 2,3,7,8-Tetrachlorodibenzofuran in Chicks. II. Effects on Drug Metabolism and Porphyrin Accumulation. Goldstein, Joyce A., McKinney, James D., Lucier, George W., Hickman, Patricia, Bergman, Hinda and Moore, John A. (1976). *Toxicol. Appl. Pharmacol.* 36, 81–92. Pure hexachlorobiphenyl (HCB) isomers induce a number of changes in parameters of drug metabolism in the chick including changes in cytochrome P-450, liver weight, and *p*-nitrophenol glucuronyl transferase, but not in testosterone glucuronyl transferase activity. The most active inducers of drug metabolism were 2,3,4,2′,3′,4′-HCB and 2,4,6,2′,4′,6′-HCB, while 2,4,5,2′,4′,5′-HCB produced intermediate effects and 2,3,6,2′,3′,6′-HCB was a poor inducer. All HCBs caused uroporphyrin accumulation and increased δ-aminolevulinic acid (ALA) synthetase activity, but only 3,4,5,3′,4′,5′-HCB, 2,3,4,2′,3′,4′-HCB, and 2,4,5,2′,4′,5′-HCB produced gross accumulation of hepatic porphyrins. Tissue HCB concentrations correlated well with hepatic effects. 2,3,7,8-Tetrachlorodibenzofuran (TCDF), a contaminant of commercial polychlorinated biphenyl (PCB) mixtures, had no effects on hepatic ALA synthetase activity, porphyrin accumulation, or glucuronyl transferase. TCDF did produce a slight increase in cytochrome P-450, but the increase was smaller than that produced by HCBs. Also, the cytochrome induced by TCDF appeared qualitatively different from the cytochrome induced by HCBs, differing by a shift in the peak of the CO-difference spectrum from 450 to 449 nm and by the ratio of absorbance at 455 to 430 nm of the ethyl isocyanide difference spectrum.

Polychlorinated biphenyls (PCBs) are industrial chemicals which are widely distributed in the environment. They have been found in tissues of wildlife and humans (Risebrough *et al.*, 1968; Biros *et al.*, 1970). Commercial mixtures of PCBs have been shown to increase liver weight, cytochrome P-450 and activity of a number of drug-metabolizing

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enzymes including glucuronyl transferase, and to produce experimental hepatic porphyria (Litterst *et al.*, 1972; Vos *et al.*, 1971; Goldstein *et al.*, 1974a).

In general, the stimulatory effect of PCB mixtures on hepatic enzymes appears to be related to the chlorine content of the biphenyl, since potency increases with increasing percent of chlorination (Litterst *et al.*, 1972). However, two PCB mixtures with identical chlorine content by weight but different isomer composition (Aroclor 1242 and Aroclor 1016) are quite different in their effects on microsomal enzymes (Goldstein *et al.*, 1975). Little work has been done thus far comparing effects of isomerically pure chlorobiphenyl compounds. Johnstone *et al.* (1974) recently compared a number of chlorinated biphenyls in the rat at a single-dose level and found 4,4-di-, 2,4,2′,4′-tetra-, 2,4,5,2′,4′,5′-, 2,3,5,2′,3′,5′-, and 2,4,6,2′,4′,6′-hexa-, 2,3,4,5,2′,3′,4′,5′-octa-, and 2,3,4,5,6,2′,3′,4′,5′,6′-decachlorobiphenyls to be extremely active inducers of a number of drug-metabolizing enzymes. However, 2,2′- and 2,4′-dichlorobiphenyl were considerably less active, as was 2,5,2′,5′-tetrachlorobiphenyl, indicating that the position of chlorines on the biphenyl is important for activity. Fujita *et al.* (1971) found 2,4,5,3′,4′-penta and 3,4,5,3′,4′,5′-hexachlorobiphenyls to be more active inducers of hepatic drug-metabolizing enzymes than 4,4′-di- and 3,4,2′,4′-tetrachlorobiphenyls, suggesting that the number of chlorines per biphenyl may be important.

Complicating the situation even more, several commercial preparations of PCBs contain traces of the highly toxic chlorinated 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Vos *et al.*, 1970). The chlorinated dibenzo-*p*-dioxins and dibenzofurans have been reported to increase both drug-metabolizing enzymes and δ-aminolevulinic acid (ALA) synthetase, the rate-limiting enzyme in heme and porphyrin synthesis (Lucier *et al.*, 1973; Poland and Glover, 1973), and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCCD) has been found to be porphyrogenic to mammals (Goldstein *et al.*, 1973). Little work has been done on the mammalian effects of TCDF.

The present study was undertaken to compare effects of several hexachlorobiphenyl isomers on a number of parameters of hepatic function in the chick, including induction of hepatic drug-metabolizing enzymes and precipitation of hepatic porphyria. These effects were compared with those of TCDF.

METHODS

Chemicals. 2,4,5,2′,4′,5′-Hexachlorobiphenyl (HCB) (>99% pure), 2,3,6,2′,3′,6′-HCB (>99% pure), and 3,4,5,3′,4′,5′-HCB (66% pure) were synthesized at NIEHS (McKinney *et al.*, 1976). The impurity in 3,4,5,3′,4′,5′-HCB was 2,3,4,5,3′,4′,5′-heptachlorobiphenyl. A second batch of 3,4,5,3′,4′,5′-HCB (99% pure) was later synthesized by McKinney *et al.* (1976) by an alternate method. 2,4,6,2′,4′,6′-HCB (>99% pure) and 2,3,4,2′,3′,4′-HCB (>99% pure) were purchased from Analabs, Inc. TCDF (88% pure) was synthesized by Dr. A. S. Kende, University of Rochester, Rochester, New York. The contaminant was 1,2,3,7,8-pentachlorodibenzofuran.

Animals. One-day-old White Leghorn cockerels were obtained from Central Carolina Farmers, Durham, North Carolina. In the first experiment, the chicks were fed a diet formulated for chick edema bioassay from Nutritional Biochemicals, Cleveland, Ohio, containing 0 or 400 ppm of each of the hexachlorobiphenyls listed above or dosed orally with 1 or 5 μg/kg/day of TCDF in 0.1 ml of acetone:corn oil (1:7). The 3,4,5,3′,4′,5′-isomer used was from the first synthesis (66% pure). Another group of chicks received 0.1 ml of acetone:corn oil (1:7). The initial number of birds per group varied from 6 to 10. Cage temperature was maintained at 30°C for 2 weeks, then lowered to room temperature (25°C) at the beginning of week 3. After 3 weeks, surviving chicks were killed by carbon dioxide anesthesia. Liver enzymes were determined on four to five animals per group, and liver porphyrins on all animals. In a second experiment, chicks were fed 0, 100, and 300 ppm of both 66 and 99% pure 3,4,5,3′,4′,5′-HCB (10 chicks per group).

Liver. A portion of the liver was excised, homogenized in ice-cold 0.2 M HEPES buffer, pH 7.4, microsomes prepared (Goldstein *et al.*, 1974a), and the latter resuspended in 0.2 M HEPES buffer for assay of glucuronyl transferase, or in 1.15% KCl containing 0.1 M K_2HPO_4-KH_2PO_4 buffer (pH 7.4) and 25% glycerol for cytochrome P-450 analysis. The resuspended pellet was stored overnight at 0°C before determination of cytochrome P-450 and ethyl isocyanide ratios.

Enzyme assays. ALA synthetase activity was assayed in whole homogenates of a second portion of liver (Goldstein *et al.*, 1974a). *p*-Nitrophenol glucuronyl transferase (Lucier *et al.*, 1971) and testosterone glucuronyl transferase activities (Lucier, 1974) were determined in Triton X-100 treated microsomes.

Microsomal cytochromes and protein. Microsomal protein was measured by the method of Lowry *et al.* (1951) using crystalline albumin as the standard. Microsomal cytochrome P-450 (Omura and Sato, 1964) and ethyl isocyanide ratios (Sladek and Mannering, 1966) were measured using a DW-2 spectrophotometer.

Porphyrins. Total liver porphyrins were determined by extraction into 0.9 M perchloric 1-1 ethanol (1:1, v/v) and measured fluorometrically with uroporphyrin as a standard (Abritti and De Matteis, 1971–1972). Liver porphyrins of some samples were extracted from 0.5 to 1 g of liver, separated by thin-layer chromatography (Doss, 1967), and quantitated as previously described (Goldstein *et al.*, 1974a).

Tissue residue analysis. Subcutaneous adipose tissue and liver samples were pooled within each treatment group, extracted, and hexachlorobiphenyl residue concentrations determined by gas–liquid chromatography with a flame ionization detector (McKinney *et al.*, 1976).

RESULTS

Chicks fed 400 ppm of 66%, 3,4,5,3′,4′,5′-HCB or dosed orally with 5 μg/kg of TCDF died before sacrifice, preventing determination of their effects on liver enzymes. Results in Fig. 1 show that all other hexachlorobiphenyl isomers tested increased cytochrome P-450 content of chick livers (4- to 14-fold). 2,3,4,2′,3′,4′-HCB produced significantly greater and 2,3,6,2′,3′,6′-HCB significantly less effects than other isomers. TCDF (1 μg/kg) increased cytochrome P-450 significantly over corn oil-treated controls (threefold), but less than all HCB isomers other than 2,3,6,2′,3′,6′-HCB. The peak of the CO-difference spectrum was shifted from 450 nm for controls to 449 nm by TCDF, but remained at 450 nm in the livers of PCB-treated animals.

Liver weight was increased significantly by all PCBs except 2,3,6,2′,3′,6′-HCB (Fig. 1). The pattern was slightly different than for cytochrome P-450, in that 2,3,4,2′,3′,4′-HCB no longer produced the greatest effects. The largest increase was produced by

2,4,6,2′,4′,6′-HCB ,which was significantly greater than that produced by 2,4,5,2′,4′,5′-HCB or 2,3,6,2′,3′,6′-HCB. The second largest increase (2,3,4,2′,3′,4′-HCB) was significantly larger than that produced by 2,3,6,2′,3′,6′-HCB. TCDF did not significantly increase liver weight.

The sequence of effects on microsomal protein were similar to those on cytochrome P-450, 2,3,4,2′,3′,4′-HCB producing the largest increase and 2,3,6,2′,3′,6′-HCB the least (Fig. 1). Only 2,3,4,2′,3′,4′-HCB, 2,4,6,2′,4′,6′-HCB, and TCDF produced significant increases. The effects of 2,3,4,2′,3′,4′-HCB were significantly greater than those of 2,3,6,2′,3′,6′-HCB or 2,4,5,2′,4′,5′-HCB.

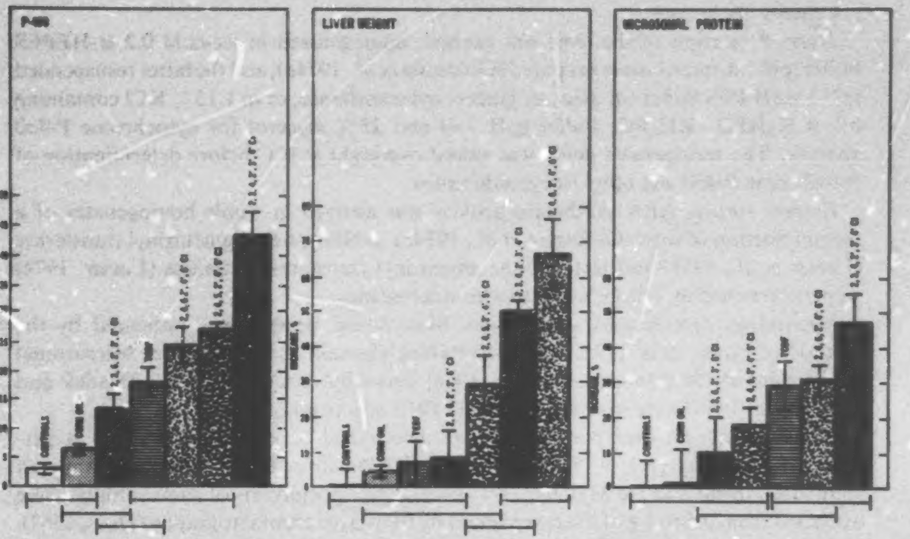

FIG. 1. Effects of purified hexachlorobiphenyl isomers and TCDF on chick livers. The hexachlorobiphenyl isomers indicated above were fed to day-old chicks at a dietary concentration of 400 ppm for 21 days. TCDF was given orally at a dose of 1 μg/kg daily for 21 days in acetone:corn oil (1:7). Controls for this group were dosed daily with acetone:corn oil (1:7). Vertical bars represent the means ± SE. Control values for liver weight were 7.3 ± 0.4 g and for liver microsomal protein were 23.9 ± 1.9 mg/g. Results were analyzed by Duncan's Multiple-Range test (Steel and Torrie, 1960), and the heavy lines below the bar graph indicate groups which do not significantly differ from each other ($p < 0.05$). Groups not underlined by the same line are significantly different.

All PCBs increased *p*-nitrophenol glucuronyl transferase activity (14- to 23-fold) (Fig. 2). Again the smallest increase was seen with 2,3,6,2′,3′,6′-HCB and the largest increase with 2,3,4,2′,3′,4′-HCB. Unlike *p*-nitrophenol glucuronyl transferase activity, testosterone glucuronidation was not affected by any of the compounds tested.

Table 1 shows the effects of hexachlorobiphenyl isomers and TCDF on the ethyl isocyanide ratios of chick liver microsomes. None of the HCB isomers had any effect on the ethyl isocyanide ratios, but TCDF did increase the ratio slightly.

As shown in Table 2, all PCBs tested produced similar increases in ALA synthetase activity (five- to six-fold), unlike the differential effects on liver weight and cytochrome

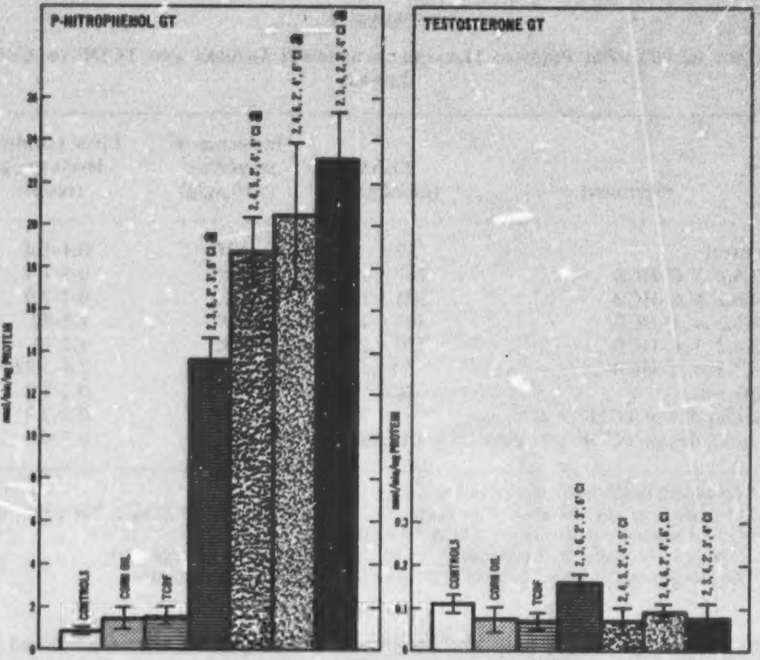

FIG. 2. Effects of purified hexachlorobiphenyl isomers and TCDF on hepatic *p*-nitrophenol and testosterone glucuronyl transferase activities. Day-old male chicks were fed 400 ppm of the above hexachlorobiphenyls or dosed orally with 1 μg/kg of TCDF in acetone:corn oil (1:7) for 21 days. Vertical bars represent the mean ± SE. [a] Significantly different from controls; $p < 0.05$ (Student's *t* test).

TABLE 1

EFFECTS OF HEXACHLOROBIPHENYL ISOMERS AND TCDF ON ETHYL ISOCYANIDE DIFFERENCE SPECTRA IN THE CHICK[a]

Treatment	455/430 ratio
Controls	0.42 ± 0.01
2,4,5,2′,4,′5′,-HCB	0.40 ± 0.04
2,3,6,2′,3′,6′-HCB	0.40 ± 0.10
2,4,6,2′,4′,6′,-HCB	0.37 ± 0.01
2,3,4,2′,3′,4′-HCB	0.43 ± 0.01
Corn oil	0.51 ± 0.07
1 μg/kg of TCDF	0.74 ± 0.06[b]

[a] Treatment is described in the legend of Fig. 1. Values represent the means ± SE.

[b] Significantly different from controls. $p < 0.05$ (Duncan's Multiple-Range test).

TABLE 2

EFFECT OF 400 PPM PURIFIED HEXACHLOROBIPHENYL ISOMERS AND TCDF ON CHICK LIVERS[a]

Treatment	ALAS (nmol/g/hr)	Incidence of porphyria[b] (>10 μg/g)	Liver porphyrin levels (μg/g) (range)
Control	55 ± 2	0/10	0.4–1.4
2,3,6,2′,3′,6′-HCB	261 ± 15[c]	0/10[d]	0.5–1.4
2,4,6,2′,4′,6′-HCB	303 ± 15[c]	0/10[d]	0.7–2.9
2,4,5,2′,4′,5′-HCB	309 ± 13[c]	3/9[d]	1.5–82
2,3,4,2′,3′,4′-HCB	250 ± 28[c]	2/8[d]	1.2–81
3,4,5,3′,4′,5′-HCB	Dead	1/7[d]	2.4–5000
Corn oil	74 ± 2	0/3	0.4–1.2
1 μg/kg/day of TCDF × 21 days	63 ± 20	0/5	0.4–2.9
5 μg/kg/day of TCDF × 21 days	Dead	0/5	0.3–0.7

[a] Treatment described in legend of Fig. 1.

[b] Proportion of animals whose liver porphyrin concentrations exceed 10 μg/g. The upper 99% tolerance levels for controls at $p < 0.01$ is 2.3 μg/g.

[c] Significantly greater than controls, $p < 0.05$ (Duncan's Multiple-Range test).

[d] Uroporphyrin detectable in all samples after thin-layer chromatography.

P-450. In contrast, TCDF produced no effect. Liver porphyrins were determined in all groups, including animals which died or were killed or moribund before 21 days. Table 2 shows the incidence of porphyria, defined as a porphyrin concentration greater than 10 μg/g of liver (control = 0.8 ± 0.1 μg/g), and the range of each group. The upper tolerance limit for 99% of control samples at $p < 0.01$ is 2.3 μg/g. Many of the animals in all groups were within the tolerance limits for control samples. However, 3/9 of the samples fed 400 ppm 2,4,5,2′,4′,5′-HCB, 2/8 of the samples fed 2,3,4,2′,3′,4′-HCB, and 1/7 of the samples fed 3,4,5,3′,4′,5′-HCB (died or were killed moribund before 21 days) were grossly porphyric. Although no animals fed 2,3,6,2′,3′,6′-HCB were porphyric, neither were any animals fed 2,4,6,2′,4′,6′-HCB porphyric. Extraction, purification, and thin-layer chromatography were performed on two to three liver samples from each treatment group. Only traces of protoporphyrin and coproporphyrin were found in control livers, but traces of 8- and 7-carboxyporphyrins were seen in all PCB-treated livers analyzed, even from those animals fed 2,3,6,2′,3′,6′-HCB and 2,4,6,2′,4′,6′-HCB. Only 8- and 7-carboxyporphyrins could be detected in porphyric livers. Porphyrin also accumulated in the crop and bones. The porphyrin in the crop was 4-, 7-, and 8-carboxyporphyrins (31, 20, and 49%, respectively), while the porphyrin in the bones was entirely 7- and 8-carboxyporphyrins (27 and 73%). None of the animals fed 1 or 5 μg/kg of TCDF showed any accumulation of porphyrins, even after extraction and thin-layer chromatography.

In the second experiment, chicks fed 0, 100, or 300 ppm of 66% pure (crude) versus 99% pure (pure) 3,4,5,3′,4′,5′-HCB died before 21 days; therefore, effects on liver enzymes were not determined. Liver porphyrins were elevated by both pure and crude 3,4,5,3′,4′,5′-HCB at both dietary levels (Table 3).

TABLE 3

EFFECT OF 3,4,5,3′,4′,5′-HEXACHLOROBIPHENYL ON HEPATIC PORPHYRINS[a]

Treatment	Incidence of porphyria[b]	Liver porphyrins[c] (μg/g)
Control	0/10	0.66 ± 0.07
100 ppm 3,4,5,3′,4′,5′-HCB(66% pure)	5/8	16.7 ± 11.2[d]
300 ppm 3,4,5,3′,4′,5′-HCB(66% pure)	7/7	200 ± 53[d]
100 ppm 3,4,5,3′,4′,5′-HCB(99% pure)	8/8	68 ± 42[d]
300 ppm 3,4,5,3′,4′,5′-HCB(99% pure)	7/7	267 ± 127[d]

[a] Two synthetic batches of 3,4,5,3′,4′,5′-HCB (66% and 99% pure) were fed to chicks at the dietary concentrations indicated for 21 days. All chicks fed 100 or 300 ppm of either batch of the HCB died before 21 days, and liver samples were taken at autopsy.

[b] Proportion of animals whose liver porphyrins exceed the upper 99% tolerance limit for controls at $p < 0.01$ (1.75 μg/g).

[c] Values represent means ± SE.

[d] Significantly greater than controls, $p < 0.05$ (Student's *t* test).

Toxicity expressed as death (groups fed 400 ppm 3,4,5,3′,4′,5′-HCB or dosed orally with 5 μg/kg/day of TCDF) or body weight loss (Fig. 3) appears unrelated to effects on the liver. 2,4,5,2′,4′,5′-HCB and 2,3,4,2′,3′,4′-HCB caused significant weight loss, while 2,4,6,2′,4′,6′-HCB produced no weight loss.

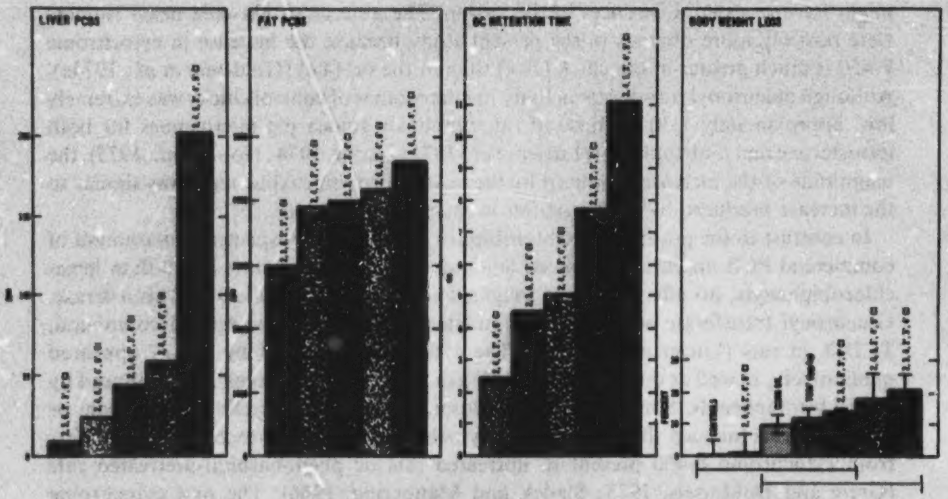

FIG. 3. Liver and fat concentration of hexachlorobiphenyls and body weight loss of chicks fed purified hexachlorobiphenyl isomers. Chicks were treated as described in Fig. 1. Values for liver and fat concentrations of PCBs are the results of duplicate analyses of pooled liver samples. Body weight loss is reported as percentage of control of body weight (203 ± 10 g). Values represent means ± SE, and heavy lines beneath bars indicate groups which do not differ significantly, $p < 0.05$ (Duncan's Multiple-Range test).

The PCB concentration of fat paralleled effects on liver weight (Fig. 3). 3,4,5,3′,4′,5′-HCB which was lethal at 400 ppm in the diet, produced the highest liver and fat PCB content. The 2,3,6,2′,3′,6′-isomer accumulated the least and produced the least liver effects. GC retention times (Fig. 3) correlate reasonably well with HCB content of fat, except for the relatively low retention time and high fat levels and biological activity of 2,4,6,2′,4′,6′-HCB.

DISCUSSION

Our results show that pure hexachlorobiphenyls alter a number of parameters of hepatic function in the chick, including cytochrome P-450, microsomal protein, liver weight, and glucuronyl transferase activity. There were distinct differences in the induction produced by the hexachlorobiphenyl isomers tested: 2,3,4,2′,3′,4′-HCB and 2,4,6,2′,4′,6′-HCB were the most active inducers, 2,4,5,2′,4′,5′-HCB produced intermediate effects, and 2,3,6,2′,3′,6′-HCB was a relatively poor inducer of the above biochemical parameters. These results show that even within the highly chlorinated hexachlorobiphenyls, position of the chlorines is important for activity. The relative inactivity of the 2,3,6,2′,3′,6′-hexa- isomer indicated that occupation of the *p*-position may be important. The work of Johnstone *et al.* (1974) also indicated that occupation of the *para*-positions may be a very important factor governing ability to induce liver enzymes, especially with PCB isomers with low chlorine content. Differences among the more potent hexa- isomers were not obvious, probably because the dose utilized produced a maximum increase. An additional lower dose of the hexachlorobiphenyls might have revealed differences in this group. The differences between hexa- isomers were possibly more obvious in the present study because the increase in cytochrome P-450 is much greater in the chick (14×) than in the rat (4×) (Goldstein *et al.*, 1974a). Although glucuronyl transferase activity in microsomes of control chicks was extremely low, approximately 1/30 of those of rat, rabbit, or guinea pig microsomes for both testosterone and *p*-nitrophenol (Lucier *et al.*, 1971; Lucier, 1974; Hook *et al.*, 1975), the magnitude of the increase produced by the hexachlorobiphenyl isomers was similar to the increase produced by PCB mixtures in rats (Goldstein *et al.*, 1975).

In contrast to the purified hexachlorobiphenyls, TCDF, a suspected contaminant of commercial PCB mixtures, produced less induction of cytochrome P-450 than hexachlorobiphenyls, no effect on liver weight, and no effect on glucuronyl transferase. Glucuronyl transferase activity was stimulated markedly by the related compound, TCDD, in rats (Lucier *et al.*, 1973). The cytochrome induced by TCDF appeared qualitatively, as well as quantitatively, different from the cytochrome P-450 induced by hexachlorobiphenyls. Similarly, administration of certain polycyclic hydrocarbons or TCDD to rats induced the formation of cytochrome P-448, a cytochrome that differs from cytochrome P-450 present in untreated rats or phenobarbital-pretreated rats (Greig and DeMatteis, 1973; Sladek and Mannering, 1966). The new cytochrome differed from cytochrome P-450 by a 2-nm shift in the peak of the CO-difference spectrum, increase in the ratio of the 455 to 430 nm peaks of the ethyl isocyanide difference spectrum, and the substrate specificity. Administration of PCBs, on the other hand, appeared to increase formation of both cytochromes P-450 and P-448 in rats: the peak of the CO-difference spectrum was shifted to 448 nm, but the ratio of the

455 nm to the 430 nm peaks of the ethyl isocyanide difference spectrum was changed to a value intermediate between those found after treatment with polycyclic hydrocarbons and phenobarbital, and there was increased metabolism of both substrates metabolized primarily by cytochrome P-450 and cytochrome P-448 (Alvares *et al.*, 1973). In the present study, only TCDF shifted the peak of the CO-difference spectrum and altered the ratio of the 455/430 nm ethyl isocyanide peaks. The apparent formation of cytochrome P-450 rather than cytochrome P-448 after administration of PCB isomers was unexpected, and may reflect either a species difference or differences between isomers. Alternately, induction of cytochrome P-448 by PCB mixtures may be a result of contamination by TCDF.

We previously reported that PCB mixtures produced hepatic porphyria, accumulation of uroporphyrins, and increased activity of ALA synthetase in the rat (Goldstein *et al.*, 1974a). Only three of the hexachlorobiphenyls tested in the present study produced an incidence of porphyria: 2,4,5,2′,4′,5′-HCB, 2,3,4,2′,3′,4′-HCB, and 3,4,5,3′,4′,5′-HCB. Interestingly, these isomers were also the most toxic, as measured by death or weight loss. It is notable that all three of these isomers had both *m*- and *p*-positions occupied by chlorines, while 2,3,6,2′,3′,6′-HCB and 2,4,6,2′,4′,6′-HCB with *p*- or *m*-positions, respectively, unoccupied were inactive. Alternatively, the nonplanarity provided by the 4 *ortho* chlorines may have decreased the porphyrogenic activity of these two compounds. However, all hexachlorobiphenyl isomers tested increased ALA synthetase and produced a slight accumulation of uroporphyrins. The time course of this disease has not been studied in chickens, but there is a lag of several weeks before porphyria and increased activity of ALA synthetase can be demonstrated in rats fed PCBs (Goldstein *et al.*, 1974a). The induction of ALA synthetase and slight increase in uroporphyrin accumulation in all chicks suggests that all hexachlorobiphenyls tested were porphyrogenic; however, there is probably a lag period in the onset of the disease, and some hexachlorobiphenyls appear more porphyrogenic than others. Reports that certain tetrachlorobiphenyl isomers did not increase ALA synthetase when administered acutely to rats (Chen *et al.*, 1973) are of doubtful significance, since similar doses of PCB mixtures did not increase ALA synthetase activity when given acutely to rats, but increased activity when fed chronically (Goldstein *et al.*, 1974a).

The presence of small amounts of TCDF in some commercial PCB mixtures (Vos *et al.*, 1970) has led many authors to speculate that the porphyrogenic effects of PCBs could be produced by contamination with TCDF. The related compound TCDD was porphyrogenic to mice at doses of 25 μg/kg/week × 4 weeks (Goldstein *et al.*, 1973), and both TCDD and TCDF have been shown to induce ALA synthetase and aryl carbon hydroxylase in the chick embryo at approx 5×10^{-11} mol/egg (Poland and Glover, 1973). The present study shows that TCDF did not produce porphyria in chicks, even at lethal doses, or increase ALA synthetase. We have also shown that TCDF does not produce accumulation of porphyrins in livers of mice at doses as high as 4 mg/kg (unpublished observations; Goldstein *et al.*, 1974b). These results suggest strongly that TCDF plays no significant role in the production of porphyria by PCB mixtures. Similarly, the relatively lesser effects of TCDF than hexachlorobiphenyls on liver enzymes make it extremely unlikely that any significant portion of the effects of PCB mixtures could be due to contamination with trace amounts of TCDF. Possibly, differences in absorption, distribution, or metabolism of TCDF versus TCDD may explain the relative inactivity of TCDF as an inducer of porphyria and drug-metabolizing enzymes in mammals and adult chickens, whereas both compounds are very potent in the chick embryo.

Tissue PCB concentrations can partially explain the differences in hexachlorobiphenyl isomers as hepatic inducers. The 3,4,5,3′,4′,5′-isomer accumulated the most in fat and liver, caused porphyria and was lethal. The 2,3,6,2′,3′,6′-isomer accumulated the least in both fat and liver, and produced the smallest increase in all parameters. However, some differences in hepatic effects (2,3,4,2′,3′,4′, > 2,4,5,2′,4′,5′) cannot be explained by liver concentrations. Concentrations of HCBs in fat correlated well with liver effects. This may suggest that lipophilicity is an important factor in hepatic potency. GC retention indices also correlated reasonably well with liver effects with the exception of 2,4,6,2′,4′,6′-HCB. This isomer and 2,3,6,2′,3′,6′-HCB both have four *ortho* chlorines which would tend to hinder rotation and make the compound nonplanar. The 2,4,6,2′,4′,6′-isomer is highly symmetrical, however, adding to its polarity. GC retention times may reflect lipophilicity with the exception of biphenyls which have four *ortho* positions occupied by chlorine. Alternatively, 2,3,6,2′,3′,6′-HCB could exist as a racemic mixture because of steric hindrance provided by the *ortho* chlorines. It is possible that one form is relatively inactive, resulting in lower activity for the mixture. Significantly, Bush *et al.* (1974) and Baker *et al.* (1974) found that tissue persistence of individual isomers in chickens fed Aroclor 1254 correlated with *para*-substitution and mixed-function oxidase activity especially for the penta- and hexa- isomer.

Tissue PCB concentrations may be an important factor in biological effectiveness. It is well known that some isomers, particularly the lower chlorinated homologs, are preferentially metabolized and excreted over highly chlorinated homologs (Weigel and Smith, 1974). On the other hand, studies with Aroclor mixtures with comparable chlorine content but differing isomer composition (i.e., Aroclors 1242 and 1016) demonstrated marked differences in biological potency despite comparable tissue concentrations (Goldstein *et al.*, 1975). The question remains as to the extent to which differences in absorption, metabolism, and distribution determine biological activity of polychlorinated biphenyl isomers. Future studies should direct further attention to the importance of the position and number of chlorines on the biphenyl nucleus in determining biological activity, and the relationship of these parameters to metabolism and tissue distribution.

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The Pesticide Problems of the Agricultural Worker*

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The pesticide exposure of the agricultural worker has formed part of the research investigation of the Community Pesticide Studies of Dade County. Health effects have been studied in the areas of acute, occupational and incidental exposure. In contrast to other areas of the United States, acute pesticide poisonings in this work group have resulted more from spray procedures and misuse rather than from crop and leaf residue intoxications. Case studies of these intoxications are presented. With regard to occupational exposure, significant cholinesterase inhibition had been noted in a group of 300 agricultural workers; increased organochlorine residues were also observed and levels were as high as pesticide formulators. These biological indices of pesticide exposure were related to SMA-12 data and blood pressures.

In stepwise regression analyses of SMA-12 data there were highly significant correlations of cholesterol with serum dieldrin levels in 277 workers; these were seen in the 0-19 and in the 20-and-over age groups. The interpretation of these and other findings are described in this report.

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The Chronic Toxicity of Hexamethylphosphoramide in Rats

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Several years ago we studied the acute and subacute toxicity of hexamethylphosphoramide (HMPA) (Kimbrough and Gaines 1966). HMPA had been suggested as a chemosterilant for houseflies (Chang et al. 1964). In our studies atrophy of the testes was observed in adult male rats fed 750 ppm (80-40 mg/kg/day) in the diet for 61-103 days. A high incidence of pneumonia was also found in the poisoned animals. This finding was further investigated (Kimbrough, R.D. and Sedlak, V.A., 1968). Corresponding observations were made by Jackson et al. (1966, 1969). Shott et al. (1971) reported a reproduction study where 2 and 10 mg/kg HMPA were given to male and female rats daily for 169 days. Reproduction was not affected but a high incidence of murine pneumonia was observed. Following our preliminary study (Kimbrough and Gaines 1966) we investigated the toxicity of HMPA in a long term study in rats with lower dietary levels. It is the purpose of this communication to report very briefly the main findings of that study.

the authors

In the study groups of 15 male conventional 6-week-old Sherman strain rats were fed HMPA in their diet at the rate of 6.25, 3.12, 1.56 and 0.78 mg/kg/day for 2 years. The diets were adjusted to give these dosage levels by frequent measurements of the food consumption. The controls consisted of two groups of 15 animals which were fed plain chow. The study was done in two parts. Each part consisted of two groups of treated animals and a group of controls as indicated in Table 1. The incidence of the leading abnormal findings made by microscopic examination are given in Table 1. Autopsy tissues were fixed in 10% buffered formalin and stained with hematoxylin and eosin.

After our Sherman strain rats were more than a year old they began to show evidence of periarteritis in different organs. The incidence of this abnormality was similar in the treated and in the control animals and was therefore not listed in the table. The incidence of lung disease primarily related to an inflammatory process was generally higher in the exposed animals than in the controls Table 1). Animals were considered to have lung disease when microscopic examination showed evidence of bronchitis, peri-bronchitis, bronchietases, bronchopheumonia, abcess formation or fibrosis. Several of these changes were usually observed in the same lung. Occasionally tumors were encountered which were not included in the tabulation of the lung disease. The tumor incidence was low and the same in all groups studied. Most of the tumors encountered were either reticulum cell or lymphosarcoma of the lungs. The incidence of kidney disease which consisted predominantly of chronic pyelone-

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phritis varied in the different groups but did not follow any particular pattern.

The rats receiving 6.25 mg/kg and a group of 15 controls were also tested for their ability to reproduce. Our results indicated that reproduction was not affected at these low dietary levels which is in agreement with the results reported by Shott et al. (1971). The rats were mated 6 and 12 months after onset of exposure to the HMPA containing diets, when they were 7 and 13 months old. The testicular atrophy observed microscopically occurred late in life and was the result of age and debilitating disease. In this study conventional rats were used and the high incidence of pneumonia in the treated groups is probably due to a potentiating effect of HMPA on Mycoplasma pulmonis infection (Overcash et al. 1972).

TABLE 1

Death Rate and Organopathology of Male Rats
Fed HMPA for 2 years

No. of Rats Studied Microscopically	Dosage Level HMPA mg/kg	No. Rats to die	Malignant tumors	Lung Disease	Chronic Kidney Disease	Testicular Atrophy
14	6.25	9	2	12	12	6
15	3.12	12	-	12	11	2
15	0	9	2	8	9	4
15	1.56	10	2	11	12	10
15	0.78	7	1	8	14	4
15	0	10	2	4	13	3

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Safety of DDVP for the Disinsection of Aircraft *

WAYLAND J. HAYES, Jr [1]

micrograms

DDVP (O,O-dimethyl-2,2-dichlorovinyl phosphate) is an organic phosphorus insecticide proposed for use in the disinsection of aircraft at vapour concentrations within the range of 0.15-0.25 μg per litre of air for 30 minutes. Safety tests have shown that men can withstand brief exposure to concentrations as high as 6.9 μg per litre and daily 8-hour exposure to concentrations as high as 0.5 μg per litre without clinical effect and with little or no depression of blood cholinesterase. It would appear, therefore, that DDVP could be safely used at the concentrations and exposure periods required for the disinsection of aircraft, though further studies will have to be carried out before this procedure can be definitely recommended.

DDVP (O,O-dimethyl-2,2-dichlorovinyl phosphate) is a direct inhibitor of cholinesterase (Durham et al., 1957). This means that it does not have to be metabolized to another compound in the body in order to produce its effects which, so far as is known, are connected with inactivation of the above-mentioned enzyme. Like most other organic phosphorus compounds, DDVP does not produce paralysis or prolonged muscle weakness even in susceptible animals (Durham et al., 1957). No other side-effects are known.

DDVP does differ from most other organic phosphorus insecticides in having a high vapour pressure so that its fumigant action is outstanding. If the supply of DDVP vapour is not sustained, the concentration falls rapidly, owing partly to mechanical loss and partly to hydrolysis by moisture in the air. If DDVP is released gradually from some porous or solid material, then (for any given rate of application) the concentration will be relatively low at first, but also relatively constant. This is illustrated by the accompanying figure, which is based on a study by Durham et al. (1959). It may be seen that application of DDVP to warehouses by sprinkling it on floors from which it gradually vaporized (open circles) produced relatively low, constant levels of DDVP in the air, so that effective concentrations were still present seven days after application. On the other hand, application of DDVP to warehouses at the same rates in the form of an aerosol (black points) produced very high levels of DDVP at first, but the concentrations fell rapidly so that effective levels lasted only three days.

The acute toxicity of DDVP is moderate. It is significantly less than that of parathion but much greater than that of malathion (Table 1). It is very nearly as poisonous when applied to the skin as when given by mouth. The onset of symptoms and their progression to death are rapid in animals that receive a fatal dose (Gaines, 1960), but animals that recover do so in a way that is correspondingly prompt.

The acute toxicity studies in animals, as well as a small number of accidents, have shown that skin contact with concentrated DDVP formulations would be dangerous.

Although flies and other disease-bearing insects can be killed at much lower concentrations provided the period of exposure is long enough, a DDVP concentration of approximately 0.15 to 0.25 μg per litre of air for 30 minutes is considered desirable for the disinsection of aircraft under practical conditions. It has recently become possible to produce this concentration in aircraft with little variation. There is no need for the crew of the aeroplane to have any contact with concentrated DDVP.

In judging the safety of using DDVP for the disinsection of aircraft, studies of the effect of DDVP vapour on man and animals are of particular interest. Table 2 summarizes the reports of several such studies, and the figure above permits a comparison of the air levels necessary for the disinsection

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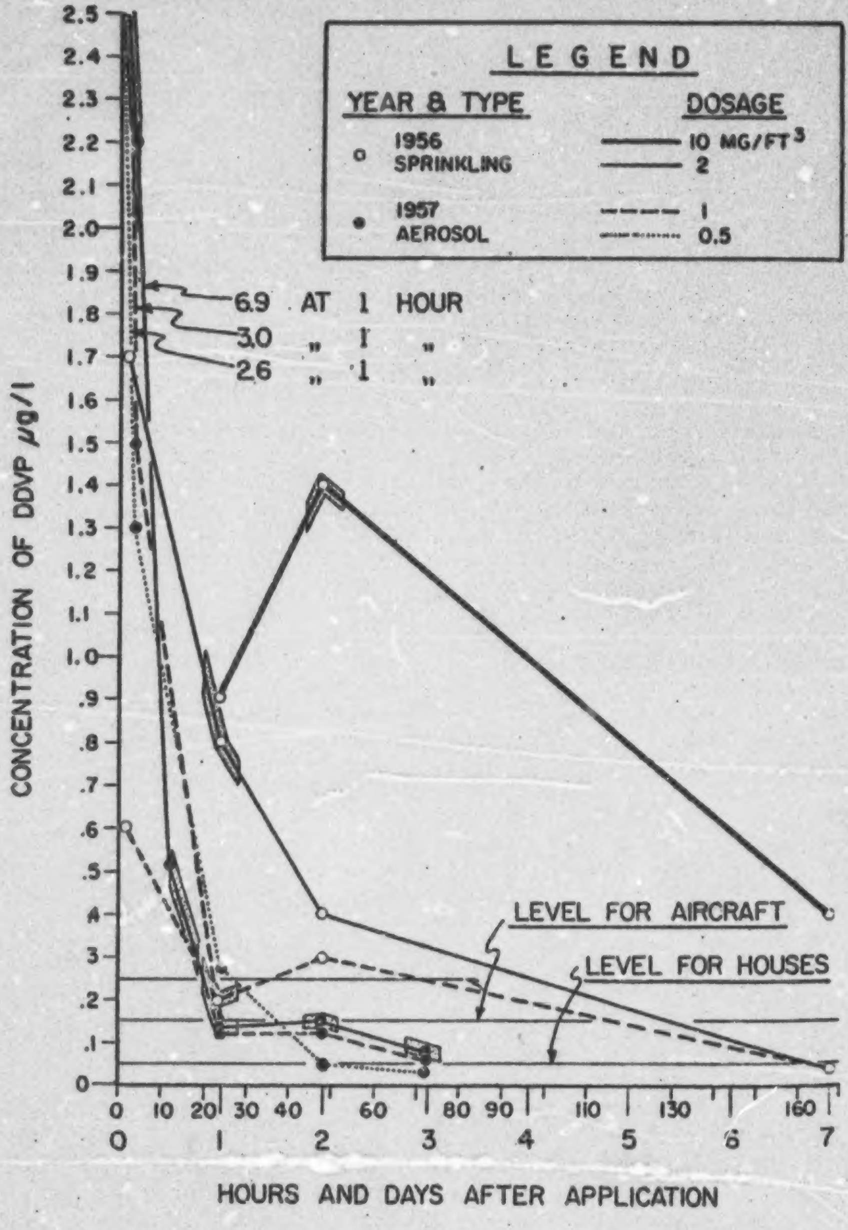
CONCENTRATION OF DDVP VAPOUR IN THE AIR OF TOBACCO WAREHOUSES FOLLOWING APPLICATION AT DIFFERENT DOSAGE RATES



The stippled areas indicate the exposure of people in certain warehouses in which the concentration of insecticide was measured. The range of concentrations of DDVP necessary for aircraft disinsection and for control of malaria mosquitos is shown for comparison. The figure is based on the original data of Durham et al (1959).

TABLE 1

ACUTE ORAL AND DERMAL TOXICITY OF DDVP, PARATHION, AND MALATHION TO MALE AND FEMALE WHITE RATS[a]

Sex	DDVP[b]		Parathion		Malathion	
	LD_{50} (mg/kg)	19/20 confidence limits (mg/kg)	LD_{50} (mg/kg)	19/20 confidence limits (mg/kg)	LD_{50} (mg/kg)	19/20 confidence limits (mg/kg)
			Oral administration			
M	80	62-104	15.0	10.2-18.5	1 375	1 206-1 568
F	56	48-65	3.6	3.2-4.0	1 000	885-1 130
			Dermal administration			
M	107	34-137	21.0	14.0-34.0	> 4 400	—
F	75	59-96	6.8	4.9-9.5	> 4 400	—

[a] Reproduced, with permission, from Durham et al. (1957).

[b] Technical grade material, prepared at the Technical Development Laboratories.

of aircraft and the levels (shaded areas) to which people were exposed in one of these studies (Durham et al., 1959).

Rats are the only animals which have been killed by DDVP vapour. A concentration in excess of 30 µg per litre was required to produce illness and exposure for more than four hours was required to produce death. Because DDVP is adsorbed on surfaces and is hydrolysed by moisture, such concentrations can be maintained only in small, closed containers in which the entire air supply is dry and is bubbled through fresh DDVP. Thus, special laboratory equipment is required to maintain fatal concentrations of the insecticide.

Miosis and a rapid drop in plasma and red cell cholinesterase have been produced in monkeys by exposure for two hours per day to concentrations of DDVP ranging upwards from 7.5 µg per litre. Even this exposure did not produce illness. Men have withstood brief exposure (30-60 minutes) to concentrations as high as 6.9 µg per litre without cholinesterase depression or any other observed effect. It is true that five men, who participated in early studies of DDVP for seven weeks and who received enough exposure to cause a moderate depression of red cell cholinesterase, complained of "lingering, unpleasant, metallic aftertaste" when working with technical DDVP or its concentrated solutions (Durham et al., 1957). But it now seems that this aftertaste was caused by dichloroacetaldehyde or some other impurity present in a relatively high concentration in the early preparation. No aftertaste has been reported in later studies or in the course of the commercial use of DDVP in tobacco warehouses for control of the cigarette beetle.

A single exposure for several hours at a concentration of about 1.0 µg per litre may produce measurable inhibition of plasma cholinesterase in man, while a slightly higher concentration is required to produce the same effect in monkeys. Higher concentrations (e.g., 1.3-1.4 µg per litre or exposure for longer periods, or both, will produce measurable reduction of both red cell and plasma enzymes in men, monkeys, and rats. Even apparently lower concentrations (0.1-0.5 µg per litre) produced recognizable depression, especially of red cell cholinesterase, in monkeys exposed for 24 hours daily for 50 days. This effect did not occur during the first 22 days of exposure. Because the air concentration of DDVP was not measured during this long experiment but was estimated from measurements during other experiments, it is impossible to exclude the possibility that the concentration of DDVP exceeded 0.5 µg per litre of air after the 22nd day of the test.

Concentrations of DDVP as high as 0.7 µg per litre (higher than those necessary for the disinsection of aircraft) have been withstood by men, monkeys, and rats for an hour or more (longer than the period required for aircraft disinsection) without any depression of blood cholinesterase or any other observed effect. The American Conference of Governmental Industrial Hygienists (1960) recently established 1.0 µg of DDVP per litre of air as the accepted

TABLE 2

EFFECT IN MAN, MONKEY, AND RAT OF EXPOSURE TO DDVP VAPOUR AT DIFFERENT CONCENTRATIONS FOR DIFFERENT PERIODS OF TIME

Species	Concentration range (μg/litre)	Duration of exposure		Results	Reference
		Hours/day	No. of days		
Man	0.1 -0.5 [a]	8	4	No effect.	Durham et al. (1959)
	0.2 -0.25	8	1	No effect.	Durham et al. (1959)
	0.25-0.88	1	4	No effect.	Witter et al. [b]
	0.3 -0.5	8	1	Questionable drop in plasma Ch.E (lowest 0.73 Δ pH/hr).	Durham et al. (1959)
	0.7 -1.0	8	1	Slight drop in plasma Ch.E (lowest 0.51Δ pH/hr).	Durham et al. (1959)
	1.3 -1.4	8	1	No further plasma depression in men previously exposed at lower levels; RBC Ch.E depression in same subjects (lowest 0.43 Δ pH/hr).	Durham et al. (1959)
	0.9 -3.5	2	4	Slight drop in plasma Ch.E (lowest 0.2 Δ pH/hr)	Witter et al. [b]
Monkey	0.03-0.27	24	4	No effect.	Durham et al. (1959)
	0.1 -0.5	24	22 50	No effect. Recognizable depression, especially of RBC Ch.E.	Durham et al. (1959)
	0.33-0.66	2	4	No effect.	Witter et al. [b]
	0.5 -1.7	24	7	Slight depression of plasma and RBC Ch.E.	Durham et al. (1959)
	1.2 -3.3	2	4	Questionable depression of plasma Ch.E.	Witter et al. [b]
	1.4 -2.0	24	7	Marked depression of plasma and RBC Ch.E.	Durham et al. (1959)
	3.0 -6.0	20	1	Marked depression of plasma and RBC Ch.E.	Durham et al. (1959)
	7.5 -17.9	2	4	Miosis and rapid drop in plasma and RBC Ch.E; no illness.	Witter et al. [b]
Rat	0.03-0.27	24	4	No effect.	Durham et al. (1959)
	0.1 -0.5	24	22 50	No effect. Recognizable depression, especially of RBC Ch.E.	Durham et al. (1959)
	0.5 -1.7	24	7	No effect.	Durham et al. (1959)
	1.4 -2.0	24	7	Slight decrease of plasma and RBC Ch.E.	Durham et al. (1959)
	>30	4.5-24	1-4	Fatal.	Durham et al. (1957)

[a] Initial brief exposures to concentrations as high as 6.9 μg per litre and later exposures to concentrations consistently below 1.0 μg per litre produced no effect on the cholinesterase level.

[b] See article on page 635 of this issue.

safe threshold limit for industrial plants, based on an 8-hour working day.

It is important to understand that cholinesterase depression is used as the most delicate available measure of the physiological effect of the organic phosphorus insecticides. There is a wide margin of safety between dosages that inhibit the enzyme and dosages that produce illness. Thus, a dietary concentration of DDVP as low as 50 parts per million (p.p.m.) produces definite lowering of the plasma and red cell cholinesterase levels of rats, yet these animals withstand a dietary level of 1000 p.p.m. for 90 days without showing signs of intoxication (Durham et al., 1957). It is well known that workers with moderate but prolonged exposure to parathion (Kay et al., 1952; Sumerford et al., 1953; Hayes et al., 1957), diazinon (Bruaux, 1957), or Dipterex (Bruaux, 1960; Lebrun & Cerf, 1960) may show marked depression of cholinesterase without any signs of illness.

Unpublished data from the Communicable Disease Center have shown that men exposed during practical tests of disinsection of aircraft (in flight and on the ground) suffered no depression of cholinesterase.

In conclusion, it appears possible to use DDVP for the disinsection of aircraft without producing any inhibition of cholinesterase in the passengers or crew. However, the possible effects of exposure on such functions as reaction time, depth perception, etc. must be determined before the procedure can be recommended. Long-term exposure tests are also contemplated as an added precaution.

RÉSUMÉ

Le seul effet connu du DDVP (phosphate de O,O-diméthyl-dichloro-2,2 vinyl) est son action inhibitrice directe sur les cholinestérases. Sa pression de vapeur est élevée, par rapport à celle d'autres insecticides organophosphorés; on peut l'incorporer dans des matériaux poreux ou solides de telle sorte que sa vaporisation puisse se faire à un taux relativement constant.

Si la toxicité buccale aiguë est modérée, la toxicité cutanée est à peu près aussi importante, au point que le contact avec des préparations concentrées serait dangereux.

On propose pour la désinsectisation des avions le DDVP à l'état de vapeur à des concentrations de 0,15 à 0,25 μg par litre d'air.

Les singes exposés à des doses de 7,5 à 17,9 μg par litre présentent une diminution des cholinestérases et du myosis, mais ne paraissent pas malades. Les hommes ont supporté de courtes expositions à des concentrations allant jusqu'à 6,9 μg par litre et par jour, des expositions de 8 heures à des concentrations allant jusqu'à 0,5 μg par litre, sans réactions cliniques et avec peu ou pas de diminution des cholinestérases sanguines. Toutes les espèces animales soumises aux essais ont résisté à des concentrations de 0,7 μg par litre pendant une heure, ou même plus, sans réactions, même sans altération du taux des cholinestérases sanguines.

Il semble donc que le DDVP puisse être utilisé sans crainte pour la désinsectisation des avions, suivant les normes résultant des essais. Cependant, on doit procéder à d'autres expériences avant de recommander l'emploi généralisé de ce produit.

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STUDIES ON THE PERCUTANEOUS ABSORPTION OF PARATHION AND PARAOXON. V. RATE OF ABSORPTION OF PARAOXON*

TORSTEN FREDRIKSSON, M.D.

One way to determine rate of percutaneous absorption is dose-response comparisons following this mode of administration and intravenous infusion. This method has been discussed in detail by Fredriksson (1) and Griesemer *et al.* (2) in connection with measurements of the rate of absorption of an organophosphorus cholinesterase inhibitor, sarin. The last-mentioned authors chose inhibition of blood cholinesterase activity in the rabbit as a basis for the dose-response comparison. It was decided to use the same parameter in connection with the two routes of administration in order to determine the rate of percutaneous absorption of paraoxon, a compound that belongs to the same pharmacologic group as sarin, but to modify the technic in several respects in order to improve the accuracy. Thus, the cat was selected as experimental animal, since this species has higher plasma cholinesterase activity than the rabbit. A higher activity makes the individual determinations of enzyme activity more accurate. Furthermore, it was decided to adjust the rate of infusion so that it corresponded as closely as possible to the rate of percutaneous absorption, since the distribution of organophosphorus compounds, their detoxification and, thus, the proportion available for enzyme inhibition is dependent on the rate of systemic administration (see review by Holmstedt, 3). Since there obviously must be a latency period before the topically applied material will reach the blood stream and produce inhibition of the enzyme activity, an adjustment of the rate of infusion so that the degree of enzyme inhibition after a given interval is the same as the degree of inhibition after the same interval following percutaneous application by necessity must result in too low values concerning the rate of percutaneous absorption. This potential error may be increased also if single measurements of enzyme activity are used because of their relative inaccuracy. Therefore, it was decided to adjust the rate of infusion so that the *rate* of enzyme inhibition was the same as following topical application. This makes corrections for the hypothetical latency period unnecessary, and the slope of the inhibition curve, determined by several measurements of activity, can be used as basis for the comparison instead of single and, therefore, uncertain activity values. Finally, in order to diminish errors due to variations in blood volume and, thus, absolute amount of enzymes present it was decided to use animals of as uniform weight as the stock permitted.

METHODS

Paraoxon (E 600, or diethyl 4-nitrophenyl phosphate) was supplied by Mallinckrodt Chemical Works, St. Louis, Mo. When tested for purity with the aid of paper chromatography (4), it was found to contain only traces of *p*-nitrophenol.

Twelve cats, weighing between 2.6 and 3.0 kg, were used. They were anesthetized with sodium pentobarbital, 25 mg per kg body weight, administered intraperitoneally. In all the animals, the trachea and one carotid artery were cannulated. Blood samples were taken from the carotid artery every 30 minutes for 4 to 5 hours. In the animals used for intravenous infusion, the left femoral vein was cannulated. In this case, the paraoxon was dissolved in propylene glycol, and the rate of infusion was adjusted to 1.0 ml per hour with the aid of an infusion pump. The concentration of paraoxon was varied between 128 and 32 μg per ml. In the animals used for percutaneous absorption, 50 μl of undiluted paraoxon was evenly distributed over 4.1 cm^2 on a clipped area on the lateral aspect of the left thigh, as described earlier (5). Plasma was separated by centrifugation, and the enzyme activity was determined with the Warburg technic, using acetylcholine as substrate. The activity values, expressed as percentage of the O-time value, were plotted in a semi-logarithmic system as ordinates

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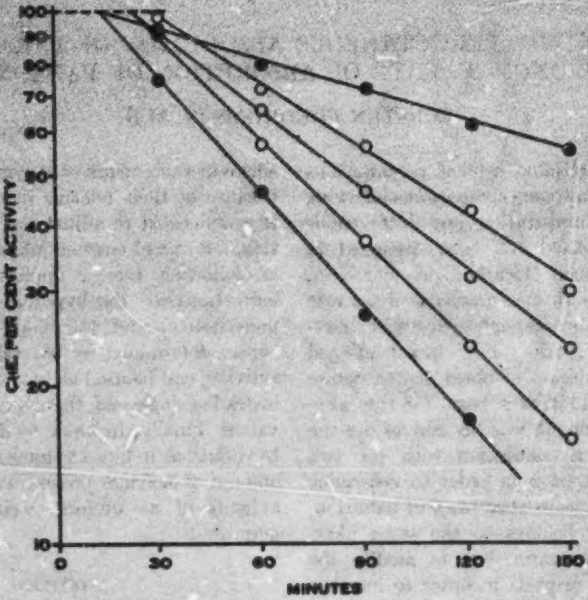

FIG. 1. Inhibition of plasma cholinesterase activity in five cats following cutaneous application (open circles) of 50 µl of paraoxon on 4.1 cm², and following intravenous infusion (filled circles) of paraoxon. The rates of infusion were 64 µg per hour (lower curve) and 32 µg per hour (upper curve). Time is in minutes after beginning of administration.

against time When plotted, the values whree. the inhibition approached asymptotic regions were omitted, and the O-time value was omitted also because the latency period must be taken into account. In order to compare the slopes of the curves, the velocity constant of the inhibition of the enzyme activity was derived from the conventional formula.

$$A_1 = A_0 \cdot e^{-k(t_1 - t_0)}, \tag{1}$$

where A_1 is the activity at an arbitrary point of time (t_1), A_0 the initial activity (at time t_0) and k the velocity constant. The constant k was calculated from the following linear transformation of equation (1):

$$k = \frac{\log A_0 - \log A_1}{0.4343(t_1 - t_0)} \tag{2}$$

TABLE 1

The first order reaction velocity constant of the inhibition of plasma cholinesterase activity following intravenous infusion and percutaneous absorption of paraoxon in the cat

Form of administration	Dosage	Velocity constant k min⁻¹
Intravenous infusion	32 µg hr⁻¹	0.0036
	32	0.0045
	64	0.0160
	64	0.0180
Percutaneous absorption	50 µl on 4.1 cm²	0.0063
	50	0.0078
	50	0.0092
	50	0.0098
	50	0.0110
	50	0.0140

RESULTS AND DISCUSSION

Cholinergic signs, such as salivation, miosis and fasciculations in different muscles, appeared occasionally in both groups of animals. The only local symptom following percutaneous absorption was fasciculations in the muscles underlying the area of application; this sign usually appeared one hour from application.

Both forms of administration resulted in inhibition curves, which followed straight lines when plotted in a semi-logarithmic system, as shown by representative curves in Fig. 1. Since it is a well known fact that the inhibition of cholinesterases by paraoxon is a first order reaction, the form of the curves means that the rate of percutaneous absorption is constant in a given skin when conditions such as temperature remain constant. This result confirms findings obtained *in vitro* (6) and *in vivo* (1) in studies of the percutaneous absorption of sarin.

All the inhibition curves obtained in the six cats used for percutaneous absorption fell within a range, which corresponded to intravenous infusion rates of 32 to 64 μg per hour. Two representative inhibition curves obtained with these infusion rates appear in Fig. 1 together with three representative curves obtained in experiments with percutaneous absorption. The velocity constants are given in Table 1. The rates of percutaneous absorption of active paraoxon into the blood stream can be calculated easily from these velocity constants. The absorption rates were approximately 0.13 to 0.26 μg min·cm², or 0.47 to 0.95 mμM min·cm². It must be remembered that these values represent only the rate of absorption of active inhibitor into the blood stream.

The disappearance rate from the topical deposit might be considerably higher than the absorption rate, since part of the material may be detoxified during diffusion through the skin. In an earlier investigation (4), it was shown that cat skin contains an enzyme that is capable of hydrolyzing paraoxon. Those results indicated that cat skin was capable of splitting approximately 30 μg paraoxon per hour and gram of tissue (1 g of cat skin equals 12–15 cm²) at 25°C, a value which does not represent optimal activity due to the thickness of the slices and to the fact that it was not measured at body temperature. In earlier experiments (1) with sarin, it appeared that approximately 90 per cent of this compound was inactivated before it reached the blood stream, and the inactivation of paraoxon might be of the same order of magnitude.

The present method of plotting the activity values makes it possible to determine the latency period mentioned earlier by extrapolation of the curves to the 100 per cent level (the broken line in Fig. 1). Following intravenous infusion, there was a latency period of 10 to 15 minutes; this period probably represents both the time necessary to build up a concentration of inhibitor high enough to produce any inactivation of the enzyme activity and the lag period due to the circulation of the blood. Following percutaneous absorption, the latency period was found to be 20 to 28 minutes. The difference between the latency period following percutaneous absorption and the one following intravenous infusion represents, thus, the actual time it takes the material to diffuse from the topical deposit to the blood stream. When curves with approximately equal slopes were chosen from both groups, the difference was found to be approximately 12 minutes. Griesemer *et al.* (2) have assumed that the latency period in the case of sarin is 15 minutes, a period of time which seems reasonable in view of the evidence given above.

SUMMARY

The rate of percutaneous absorption of paraoxon (E 600, or diethyl 4-nitrophenyl phosphate) has been determined by dose-response comparison following this mode of administration and intravenous infusion in the cat. The rate of inhibition of plasma cholinesterase activity was chosen as the parameter. The enzyme activity values were plotted in a semi-logarithmic system as ordinates against time. Under the conditions of the study, the rate of percutaneous absorption was found to be constant in a given skin. The rate varied between 0.13 to 0.26 μg min·cm² (0.47 to 0.95 mμM min·cm²). These values represent only the absorption of active material into the blood stream. The disappearance rate from the topical deposit may be considerably higher, since there are enzymes present in cat skin capable of hydrolyzing paraoxon. By extrapolation from the activity curves, it was possible to determine the time required for paraoxon to diffuse from the topical deposit to the blood stream. This latency period was found to be approximately 12 minutes.

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PESTICIDES PROGRAM
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TOXICITY OF HEXAMETHYLPHOSPHORAMIDE IN RATS

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CHANG *et al.*[1] reported that hexamethylphosphoramide (HMPA), an analogue of tepa, is an effective chemosterilant for houseflies. They emphasized that it has a low toxicity to mammals, and also that it has a wide margin of safety for flies between a lethal dose and a dose effectively sterilizing the males. Another advantage claimed for HMPA is that it is not an alkylating agent as, for example, are the ethyleneimine compounds which are also now being considered as chemosterilants. In view of the reported low acute toxicity of HMPA to mammals and its value as an insect chemosterilant, we undertook investigations of its toxicity to rats.

Male and female Sherman strain rats were used, and technical grade HMPA was either dissolved in water and given by stomach tube (while the controls were dosed with tap water) or mixed into ground Purina chow used as food. LD_{50} values for HMPA were determined according to the procedure developed by Gaines[2] for other pesticides; calculations were made according to the method of Litchfield and Wilcoxon[3]. All rats had free access to Purina chow and water. For white blood cell counts, blood was obtained by tail clipping. Complete autopsies were undertaken with most animals. Tissue for microscopic study was fixed in 10 per cent neutral formalin, and routine staining procedures were performed with haematoxylin and eosin. The left testis and epididymis were removed from a number of experimental male rats, which had been repeatedly dosed, and from a corresponding group of controls. This procedure was carried out with the animals under sodium pentobarbital anaesthesia 38–45 days after onset of exposure; microscopical examinations were then performed. These animals were then allowed to live for an additional 52 days.

The LD_{50} values for HMPA given by oral and dermal routes to adult rats are shown in Table 1. The compound when given by the oral route was more toxic to male rats than to females. The acute toxicity of HMPA by the oral route in male rats was less than that of the alkylating agents, apholate, metepa and tepa, by a factor of about 19 or greater. Gaines and Kimbrough[4] reported oral LD_{50} values of 98, 136 and 37 mg/kg, respectively, for the latter three compounds. Owing to considerable variation in the mortality among rats given single doses of HMPA by the dermal route, the LD_{50} could only be estimated. The main clinical symptoms observed in acutely poisoned rats were involuntary urination, mild muscle fasciculation, convulsions and bloody urine. (Large numbers of intact red blood cells were observed in the urine when it was investigated under the microscope.) Because HMPA is an organic phosphorus compound and because muscle fasciculation was observed, the blood cholinesterase level was determined titrimetrically in six adult male rats. When each animal was used as its own control, the change in plasma enzyme activity varied little (−3 to −42 per cent) at the time of most severe illness—about 3 h after an oral dosage of 2,000 mg/kg. Five hours after this dosage, there was some return to normal (+8 to −19 per cent). There was no significant change in red cell cholinesterase at any time.

Table 1. ACUTE TOXICITY OF HMPA IN ADULT SHERMAN STRAIN RATS

Route	Sex	LD_{50} (mg/kg)	19/20 conf. limits (mg/kg)	Lowest dose (mg/kg)	
				Tested	Lethal
Oral	M	2,650	2,325–3,021	500	2,000
Oral	F	3,360	2,754–4,099	2,000	2,500
Dermal	M	3,500–4,500*	–	3,000	3,500
Dermal	F	3,500–4,500*	–	3,000	3,500

* Estimated.

A dose of 2,200 mg/kg was given to ten adult male rats. The total white blood cell and differential counts were determined before, 3 days after, and 1 week after treatment. The white blood cells did not appear to be affected. The only animals that showed a drop in total white blood cell count were 6- to 7-week-old male rats which had been treated daily with 400 mg/kg HMPA by stomach tube for 30 days. Their average count was 8,820 white blood cells/mm³ blood (range: 5,500–13,050); the total white blood cell count of ten controls of the same age averaged 16,670/mm³ blood (range: 10,600–23,050). The lowest single dose which resulted in a loss in weight in males was 1000 mg/kg; the lowest repeated dose with this effect was 100 mg/kg/day.

In order to determine acute tissue changes, six adult male rats that had been given a single dose of 2,500 mg/kg HMPA were killed when moribund (3 days after poisoning) and their tissues examined. Two instances of necrotizing cystitis and four instances in which the tubules of the kidneys contained red blood cells and hyaline casts were recorded; these findings are consistent with the haematuria mentioned earlier. Intra-alveolar haemorrhage and bronchiectasis were observed in three rats. The spleen was smaller than normal in all animals. Even among the six rats killed only three days after a lethal dose, the testes of two of them showed a reduced number of sperms and spermatids, and occasionally multinucleated cells were observed. As will be described later, the testis proved to be the organ most consistently affected by single or repeated doses of HMPA, but other organs were also affected in some animals. For example, repeated dosing produced bronchial pneumonia with the formation of abscesses in the poisoned animals. The pneumonia was not related to the stomach tubing (Table 2), as it also occurred in the animals which received HMPA in their diet but not in the animals which received only water by stomach tube. It is possible that HMPA affects the reticulo-endothelial system and thus lowers the resistance to infection, as many poisoned animals showed very small atrophic spleens. The lymph nodes of these animals, on the other hand, were normal or enlarged.

In a further investigation of the effect of HMPA on the testes, additional groups of adult male rats were killed about 40 days after receiving single doses of varying amounts of HMPA by stomach tube. The lowest single dosage damaging the testes was 1,000 mg/kg; a single dosage of 500 mg/kg produced no effect. The lowest dosage given repeatedly by stomach tube to rats in which the testes were examined microscopically was 100 mg/kg/day, and this dosage caused testicular atrophy (see Table 2). When given in the diet at a level of 750 p.p.m., the daily intake, ranging from 80 to 40 mg/kg, produced partial or complete atrophy (Table 2). The testes were obviously smaller in living rats even when microscopic study revealed only partial atrophy. However, in a preliminary breeding experiment, it was found that when HMPA was administered by stomach tube at a rate of 25 mg/kg/day for 56 days it did not affect the fertility of male rats during that period, and the testes of the living animals appeared to be normal in size.

Microscopic examination of the testes, 36–41 days after a single dosage of 1,000 mg/kg or more, indicated that at

Table 2. Pneumonia and Testicular Atrophy in Male Sherman Strain Rats Caused by HMPA

Dosage level (mg/kg)	No. of doses Stomach tube	Diet	Days between last dose and pathological examination	Total examined	Number of animals Killed	Died	With pneumonia	With partial or complete testicular atrophy
2,500	1		36–41	9	9	0	2	9
1,000	1		36	5	5	0	3	3
500	1		36	6	6	0	1	0
0	1		36	6	6	0	0	0
[illegible]	30–60		0	8	2	6	2	8
[illegible]	33–60		0	5	3	2	3	5
100	47		0	4	surgery*	0	–	4
	34–60		0	10	9	1	5	10
0	46		0	4	surgery*	0	–	0
	36		0	10	10	0	0	0
80–40†		45	0	3	surgery*	0	–	3
		61–105		11	8	3	8	11
0		44	0	2	surgery*	0	–	0
		105	0	5	5	0	0	0

* Left testis was removed under sodium pentobarbital anaesthesia. The same animals as well as others in the group that did not undergo surgery are included in the autopsy material.

† The dietary level of HMPA was 750 p.p.m. In the first few weeks the animals ate about 110 g/kg/day so that the intake of HMPA was about 80 mg/kg/day; later as the rats matured the average food intake dropped to 55 g/kg/day and the HMPA intake to about 40 mg/kg/day

this time the spermatids are the cells of the seminiferous epithelium most affected by HMPA, but the effect is not uniform throughout the testes. Particularly after repeated dosing at or above the rate of 80–40 mg/kg/day, some tubules of some of the testes still contained normal or almost normal seminiferous epithelium while others were devoid of it or showed only a few atypical cells. We have called this condition 'partial atrophy', as in complete testicular atrophy (Table 2) all tubules showed loss of the seminiferous epithelium except for a few remaining spermatogonia. Similar pictures of partial or complete atrophy were also observed with metepa in an experiment conducted earlier[4].

A single dose of 2,500 mg/kg was given to six females. No effect on the female reproductive organs was observed when the animals were killed 36 days later.

HMPA was given to ten female rats by stomach tube at a rate of 200 mg/kg/day. The dosing began 7 days before mating to undosed males and continued until the twentieth day of pregnancy. The females were then killed and their uteri opened. The offsprings and placentae were weighed and examined grossly; the foetuses were fixed in Bouin's solution, and cross-sections were examined later under the dissecting microscope. Abnormalities were not detected in the offsprings of either the rats dosed with HMPA or eight female control rats that had been dosed daily for the same period with tap water. The weight of each foetus, the weight of each placenta, the number of animals per litter, and the number of resorption sites in the treated animals did not differ statistically from the controls.

A dosage of 100 mg/kg/day of HMPA is about 4 per cent of the acute oral LD_{50}. In earlier investigations in which metepa was administered by stomach tube, the lowest dosage level that caused partial testicular atrophy in some rats after 55 days was 10 mg/kg/day; this represents about 7 per cent of the acute oral LD_{50} dose. Metepa was found to be more toxic to rats when given daily by stomach tube in water than when given as a component in the diet—10 mg/kg/day by stomach tube had about the same effect as 76 mg/kg/day in the diet[4]. On the other hand, HMPA had about the same effect whether it was given in the diet or by stomach tube (see Table 2)—probably owing to its greater stability. Because of its stability, HMPA might present a greater problem as a residue than the ethyleneimines, which deteriorate rapidly, although both kinds of compound have a cumulative effect on the testes.

These data show that HMPA has a very specific effect on the testes of rats when fed at a dietary level of 750 p.p.m. which may be compared with a level of 2,500 p.p.m. in food necessary to cause 100 per cent sterility in male flies[1]. This comparison shows that the safety of any future use of HMPA must be based on its moderate toxicity to mammals and on limitation of their contact with it rather than on any important difference in the inherent susceptibility of mammals and insects to the compound.

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STOLEN PESTICIDES*

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Stolen Pesticides?

Since the introduction of DDT during World War II, a bewildering variety of extremely toxic pesticides have been developed and utilized in ever increasing amounts. Concomitantly, morbidity and mortality from pesticide poisoning have risen steadily. Two thirds of all pesticide poisoning cases in South Carolina occur in children.[1] The practitioner may feel insecure when confronted with poisoning emergencies, and he must depend upon the family for an accurate exposure history. This article is intended to alert the physician to the common inadequacy of poison histories and to stress the need for thoughtful clinical evaluation of the patient.

Case Reports

Case I. A pair of 2-year old girls were playing on a lumber pile in their rural backyard. While walking to the house with her mother, one child suddenly "rolled her eyes back." Thus began a series of convulsions that ended with the child's demise an hour later while en route to the emergency room. The other child vomited, was observed at the emergency room for "bean poisoning" and released after four hours. On home investigation several cupfuls of dust were found on the lumber pile. Dust analysis showed 33% aldrin, a potent chlorinated hydrocarbon; dieldrin, a metabolite of aldrin was found in the plasma (0.017 ppm) of the surviving child. Neither family nor friends would admit knowing its source. The family had no garden. The lumber pile had been present for many months and no poison had been noted when it was covered with tar paper three days before the incident. One family member had worked for a fertilizer plant where aldrin was an additive.

Case 2. Pest control operators might be expected to furnish accurate information on the poisons they use and to observe necessary storage precautions. A 16 month old child became comatose within five minutes after having had "turpentine" from an unlocked container spilled on his head by his three year old brother. He was washed immediately by his mother. When he was seen in the emergency room twenty minutes later, he was cyanotic. There were no respirations and only a feeble pulse of 20. Atropine was administered for pulmonary congestion and possible organophosphate poisoning, causing marked clinical improvement. The RBC cholinesterase was 0.83 um/min/ml. (normal 11.1-16.0). Protopam chloride (pralidoxine chloride, 2-PAM) was given immediately but because of the apparent cerebral anoxia, the child remained stuporous and vegetative after several months. The father was first located after the initial atropine treatment.

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Being a recent pest control employee, he was familiar with pesticides and stated that the poison was diazinon. It had been bought from a store for garden usage. Analysis of the container's content showed 25% DDT and 8.33% parathion, a much more potent organophosphate cholinesterase inhibitor than diazinon.

Comment

The average physician may not be able to classify pesticides into the two most common groups, i.e. organophosphate cholinesterase inhibitors and relatively less toxic chlorinated hydrocarbons. Although there are other classes of pesticides such as carbamates, the organophosphates and chlorinated hydrocarbons are by far the most significant source of pesticide poisoning in South Carolina along with arsenic, a common ingredient of many herbicides. The following table shows the oral and dermal LD_{50}* for the more common pesticides in South Carolina.

Acute Oral & Dermal LD_{50}*
(Male & Female White Rats)

Compounds	Dermal LD_{50} (mg/kg) Males	Dermal LD_{50} (mg/kg) Females	Oral LD_{50} (mg/kg) Males	Oral LD_{50} (mg/kg) Females
A. Organophosphates				
Methyl parathion	14	24	67	67
Parathion	13	4	21	7
Phosdrin	6	4	5	4
Guthion	13	11	220	220
Diazinon	108	76	900	455
Malathion	1375	1000	>4444	>4444
B. Chlorinated hydrocarbons				
DDT	113	118	2510	2510
Aldrin	39	60	98	98
Toxaphene	90	80	1075	780

*Lethal dose required to kill 50 per cent.

The alert physician should attempt to discover the concentration and form of the suspected agent, its dermal and oral LD_{50}, its toxic signs and symptoms, and the apparent route of exposure. If the clinical picture does not correlate, these factors should each be reconsidered. For example, the spilling of diazinon on the head of even a young child does not produce the rapid coma seen in Case 2. One must look for a more potent poison or oral ingestion. If oral ingestion is suspected, stomach lavage is obligatory as is more prolonged vigorous treatment with atropine and 2-PAM because continued irregular absorption from the gut may occur.

It is critical that physicians consider misinformation in poisoning cases that appear atypical. When a pest control operator supplies misinformation in his own son's poisoning, one finds it difficult to consider other testimonies too seriously. Stolen concentrated forms of pesticides are a recurrent, particularly serious source of poisonings.

In all poisoning cases, especially those with very toxic compounds, the physician should also seek to document the type of poison. The family should be instructed by the physician to bring the suspected agent and its container as soon as possible. Any tampering with the original labeled products by the family should be noted because unique potent formulations may have been prepared. If doubt exists, laboratory identification can be made by sending the pesticide to the South Carolina Community Pesticide Study located at the Medical College in Charleston. Analysis is free and information on current treatment is available.

Summary

Pesticide poisonings now constitute a significant morbidity and mortality problem in South Carolina. Misinformation about the source and type of poison is common, frequently because the poison is stolen. Physicians must acquire and study all available pertinent information to insure proper treatment of the patient.

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TOXICOLOGY PROGRAM
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Tissue Distribution of P^{32}-Labeled Parathion

Autoradiographic Technique

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AND
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ATLANTA

The distribution of organophosphorus cholinesterase inhibitors, such as disopropyl fluorophosphate (DFP), sarin,* and parathion in mammalian tissues has mainly been studied indirectly, i.e., by following the degree of enzyme inhibition in various organs and body fluids or by the development of certain cholinergic symptoms.[1] From such investigations[2-4] it has generally been concluded that compounds highly soluble in lipids are prone to produce rapid inhibition of brain cholinesterase, and, therefore, central symptoms, while inhibitors that are more water-soluble tend to give predominantly peripheral effects. However, the actual distribution of the inhibitors may not necessarily follow the pattern indicated by the enzyme inhibition, since the affinity of the compounds for a tissue, as such, might differ from that for the enzymic sites of the same tissue.

Those cases in which more direct methods[5,6] have been used to follow the distribution of organophosphorus compounds have mainly been limited to demonstration of the materials in a few tissues or body fluids, giving no detailed information about the body as a whole.

The autoradiographic method developed by Ullberg,[7,8] i.e., sectioning of whole mice at low temperature with the thin sections attached to transparent cellulose tape, offers extraordinary possibilities for determining the actual and total distribution of various therapeutics and toxicants. It was decided, therefore, to use this method to follow the tissue distribution of P^{32}-labeled parathion following subcutaneous injection, even though it was realized that this intricate technique involves additional problems in the present case. Parathion has a relatively high toxicity by this route, and the doses necessary to produce good autoradiograms are in the lethal range. Therefore the distribution could be followed for only 4 hours after injection, since the experimental animals seldom survived longer than this. It was decided not to counteract the toxicity with atropine and enzyme reactivators, as such treatment influences the metabolism of the compound and thus also its distribution.[9] Another problem involved is the slow release of parathion from the subcutaneous tissues[10] so that part of the dose remains at the point of injection and produces a pronounced shadow on the autoradiograms. Furthermore, parathion is metabolized comparatively rapidly; thus, the autoradiograms will show the distribution of the metabolites also, and the identity of all of

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* Nerve gas described as isopropoxymethylphosphoryl fluoride.

them is not known with certainty.[9,11] This latter problem is discussed below.

Methods

P^{32}-labeled parathion (E 605, diethyl 4-nitrophenyl thionophosphate) with an initial specific activity of 1.75 mc. per millimol was obtained from Volk Radiochemical Company, Chicago. The purity of the compound was tested with paper chromatography by the method of Metcalf and March[19] in a slightly modified version.[20] No contaminants could be detected by testing for nitrophenol groups. However, autoradiograms of the paper strips revealed minute traces—considerably less than 0.1%—of an unidentified compound, probably an isomer.

The active compound was emulsified in 10% ethanol with 2% of a surface-active agent, Triton X 100, manufactured by Rohm and Haas Co., Philadelphia. Two series of 5 mice of both sexes were used. The body weight varied between [illegible] and 10 gm. The animals were injected subcutaneously in the back, the dose being 0.03 mg. of parathion (corresponding to approximately 0.03μc.) per gram of body weight. The injected volume was 0.05 ml. per gram of body weight. The animals were killed by immersion in liquid nitrogen at 30 minutes and at 1, 2, 3, and 4 hours after the injection. They were immediately transferred to a freeze room (—10 C), mounted, and sectioned according to Ullberg.[7] The 100μ sagittal sections were dehydrated at the same temperature for 48 hours in small sealed boxes containing calcium sulfate. Still in the freeze room, they were exposed for 10-20 days to Eastman-Kodak No-Screen Medical X-Ray Film or Eastman-Kodak Autoradiographic Plates Type A, which gave somewhat higher resolution. The autoradiograms were developed with Eastman-Kodak D 19 developer for 5 minutes at 21 C, transferred to an acid stopping bath, and fixed for 30 minutes with Kodak fixing bath F5.

Results

The results are illustrated by Figures 1 and 2. The injected material was absorbed very slowly from the subcutaneous deposit, where a major part of the radioactivity remained throughout the period of observation.

The level of radioactivity in the major blood vessels and the heart chambers was low in all the autoradiograms, but the material had accumulated particularly in salivary glands, cervical brown fat (hibernating gland), liver, kidney, and adipose tissue. Fairly high activity was found in gastric and intestinal walls, thyroid, spleen, and lungs. The activity was somewhat lower in the central nervous system, musculature, and bone marrow, while very low or no activity could be demonstrated in thymus, intestinal lumen, bile, adrenal glands, and skin. There were no obvious sex differences.

There was a rapid accumulation of radioactivity in the salivary glands and the

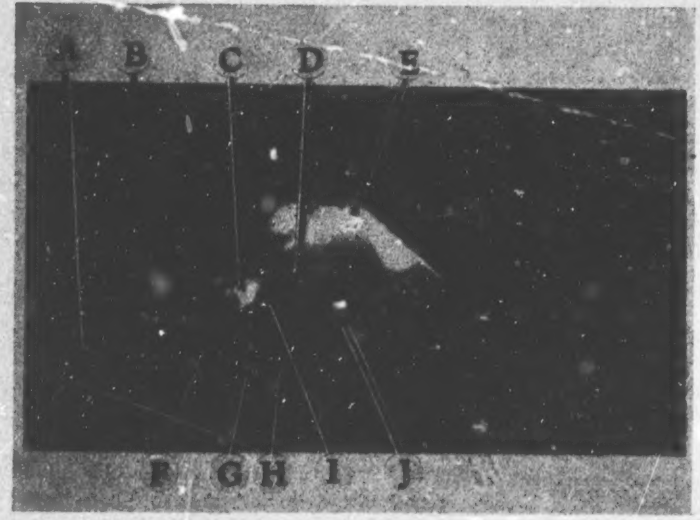

Fig. 1.—Autoradiogram showing the distribution of P^{32} in a mouse 30 minutes after subcutaneous injection of P^{32}-labeled parathion. White areas denote high P^{32}-content. *A*, eye. *B*, brain. *C*, brown fat. *D*, liver. *E*, deposit. *F*, salivary gland. *G*, thymus. *H*, blood in heart. *I*, lung. *J*, intestine.

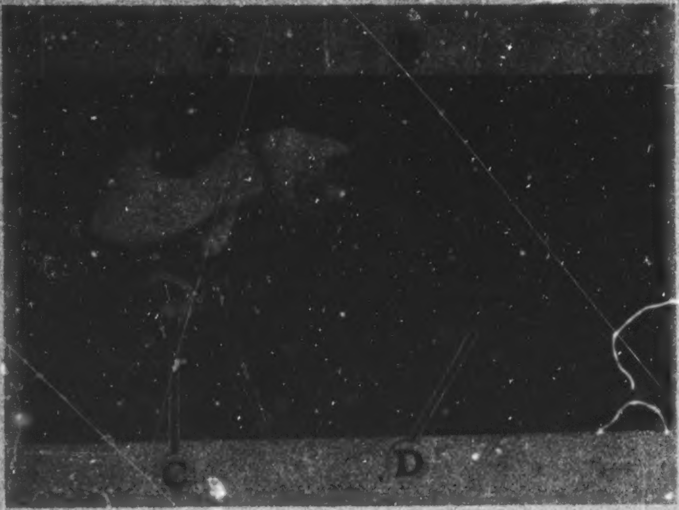

Fig. 2.—Autoradiogram showing the distribution of P^{32} in a mouse 3 hours after subcutaneous injection of P^{32}-labeled parathion. Note high activity in renal pelvis. *A*, liver. *B*, kidney. *C*, salivary gland. *D*, artefact.

cervical brown fat, and these structures contained considerably more activity than any others during the whole period of observation. The liver also showed a rapid uptake of activity, but there was no evident increase with time. In the kidney, there was an initial concentration of the activity in the cortical region, and one hour after injection there was high activity in the renal pelvis and noticeable activity in the bladder. The activity in the bladder was high at the end of the period of observation. Adipose tissue, e.g., around the eye, in the mediastinum, and paracostally, showed high and progressively increasing activity. Both gastric and intestinal walls showed a fairly high activity during the whole experiment, and there was no evident difference between these 2 structures. The central nervous system had, as compared with other structures, a low content of activity, which was very evenly distributed. There was no evident predilection for any structures, such as the cerebral cortex, the basal ganglia, the cerebellum, the medulla, or the spinal cord.

Comment

It is a well-known fact[11] that even highly purified parathion contains various contaminants, mainly because isomers are easily formed. The most important contaminant is usually O,S-diethyl 4-nitrophenyl phosphate, but traces of other isomers and of diethyl 4-nitrophenyl phosphate (paraoxon) have also been found. In the present sample of P^{32}-labeled parathion, an unidentified phosphorus-containing contaminant was demonstrated, but the presence of this presumed isomer is probably of minor or no importance for the evaluation of the present results since the amounts found were very minute.

The injected material, however, will undergo important and fairly rapid chemical changes upon its absorption into the blood stream,[9] and the possibility that such changes occur even prior to its release from the subcutaneous tissues cannot be excluded. Parathion is readily oxidized to form paraoxon, an enzymic activation which occurs mainly in the liver, but also in other tissues, e.g., the intestinal wall.[14] The paraoxon formed will take part in at least 2 different reactions. It will inhibit cholinesterases by phosphorylation of the enzyme, resulting in the release of *p*-nitrophenol. According to a recent theory[15] it is also possible that one of the ethoxy groups is split off in the reaction. As the phosphorylated enzyme is subject to both enzymic and nonenzymic hydrolysis there might thus be a release of 2 different phosphorus-containing residues. However, this spontaneous reactivation of the enzyme is rather slow. The other reaction involving the formed paraoxon is its hydrolysis by phosphorylphosphatases resulting in the formation of diethyl phosphoric acid. Parathion itself will presumably also be subject to the same type of reactions[9] with subsequent release of corresponding S-analogues. Furthermore, isomerization might occur in vivo, and it is also known that the nitro group is reactive.[11] It is thus evident that ultimately the injected parathion will be partly transformed into a wide variety of phosphorus-containing metabolites, all of which may contribute to the autoradiographic picture.

The fairly high radioactive uptake of the liver is reasonable in view of the fact that the transformation of parathion into paraoxon takes place mainly in this organ. It is also interesting to note that the intestinal wall, which is capable of oxidizing parathion,[14] contains relatively high activity. This finding might also explain why increased peristalsis and abdominal cramps are very early signs of systemic absorption of parathion.[1]

The very high affinity of parathion or its derivatives for the salivary glands helps to explain the clinical observation that profuse salivation is one of the earliest signs of systemic intoxication with parathion and the fact that this compound frequently produces morphological changes in these organs in cases of accidental poisoning.[16]

Parathion, its isomers, and paraoxon are readily soluble in lipids.[11] In view of this, accumulation of radioactivity in adipose tissue and cervical brown fat is reasonable. Similarly, the central nervous system could also be expected to show a high uptake of radioactivity due to its lipid content. This has not been the case in the present series of experiments. Evidently the circulating P^{32}-containing material has lower affinity for the central nervous system than for other tissues with a high lipid content, or the penetration into nervous structures might be restricted. This may explain why central symptoms are late manifestations of parathion poisoning.

In spite of the high uptake of the liver, there are no signs of any excretion of labeled material in the bile, nor is there any excretion via the intestinal mucosa. Instead, the P^{32}-labeled material is eliminated by the kidneys. Due to solubility properties, there are reasons to believe that the major part of the material appearing in the urine consists of breakdown products, most of which are readily soluble in water.

From the present findings it is evident that the actual tissue distribution of parathion and its metabolites does not necessarily follow the pattern expected from studies of enzyme inhibition. Parathion thus produces pronounced depression of cholinesterase activity in brain and blood, while in the present investigation only low radioactivity could be demonstrated in these sites.

The affinity of parathion and its breakdown products for certain organs and tissues might suggest new possibilities for early diagnosis of exposure to this insecticide. Perhaps such material could be demonstrated in saliva, and this might result in a useful laboratory test for recognizing systemic absorption.

Summary

The tissue distribution of P^{32}-labeled parathion (E 605, diethyl 4-nitrophenyl thionophosphate) has been investigated by means of an autoradiographic technique applied to sections of whole mice. The animals were injected subcutaneously and were killed at various intervals up to 4 hours after injection.

It was found that the material was absorbed very slowly from the subcutaneous deposit. The level of radioactivity in blood was low during the whole period of observation. However, the labeled material accumulated in various organs and tissues. The highest activity appeared in the salivary glands and cervical brown fat (hibernating gland). Liver, kidney, and adipose tissues showed high uptake of radioactivity, and fairly high activity was found in gastric

and intestinal walls, thyroid, spleen, and lungs. Less activity was noted in the central nervous system, musculature, and bone marrow. The labeled material was mainly excreted by the kidneys and not in bile or via the intestinal mucosa.

The results have been discussed with special regard to the formation of P^{32}-containing metabolites and breakdown products. The relationship between the distribution of the material and the sequence in which systemic symptoms appear in parathion intoxications has been pointed out. The fact that the actual tissue distribution of a cholinesterase inhibitor need not necessarily follow the pattern indicated by the enzyme inhibition has been stressed.

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STUDIES ON THE PERCUTANEOUS ABSORPTION OF PARATHION AND PARAOXON

I. Hydrolysis and Metabolism Within the Skin

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During the last decade, parathion has been the most widely used organophosphorus insecticide. It has proved to be extremely valuable for crop protection and useful in combating communicable diseases. However, the wide spread use of this compound has also resulted in a great number of accidental intoxications, many of which have been lethal. The dermal route of absorption is usually thought to be important in such cases (for complete bibliography up to 1958 regarding percutaneous intoxications and relevant experimental studies, see Fredriksson, 1958). However, previous experimental studies on the percutaneous absorption of parathion have been limited almost exclusively to LD_{50}-determinations in various species. It was therefore decided to perform a series of investigations covering such subjects as metabolism within the skin, penetration into the skin, rate of percutaneous absorption, and decontamination of the skin surface. In this series paraoxon also has been included, partly because parathion is transformed into this compound *in vivo*, and partly because of the need for a reference substance.

Percutaneous absorption of organophosphorus cholinesterase inhibitors, such as DFP, sarin, parathion and paraoxon, involves special theoretical problems due to the fact that many of these compounds are readily hydrolyzed or in other ways metabolized in the tissues of the body. The immediate implication of this is that a percutaneously absorbed inhibitor might be inactivated or changed in other ways before it reaches the blood stream, if such metabolic processes occur within the cutaneous tissues. Thus, it has been shown by *in vitro* studies that guinea pig skin contains an enzyme, belonging to the group of phosphorylphosphatases, that is capable of hydrolyzing sarin and two closely related compounds; and studies in the cat *in vivo* also suggest that there is hydrolysis of sarin during its absorption through the cutaneous tissues, Fred-

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riksson, 1958. The last mentioned study indicated that a major part of the material was inactivated before it entered the blood stream.

When percutaneous absorption of parathion is considered, the possible hydrolysis within the skin must be taken into account, even though there is only indirect evidence of hydrolyzing enzymes in mammalian tissues, Casida, 1956. Parathion also might be subject to another metabolic process in the cutaneous tissues, namely its oxidation to paraoxon, an enzymic activation that occurs mainly in the liver, but also in several other organs, Kubištova, 1959. However, skin has never been tested for the presence of this enzyme. A transformation of parathion into paraoxon within the skin might have two different toxicological implications. If this more toxic metabolite is formed within the skin during percutaneous absorption of parathion, and if it undergoes no further change, this portal of entry might be particularly dangerous. However, paraoxon is readily hydrolyzed by phosphorylphosphatases, and the presence of such enzymes in the skin might instead decrease the toxicity of percutaneously absorbed parathion and, of course, paraoxon.

It was, therefore, decided to investigate in various species the possible occurrence of skin enzymes capable of metabolizing parathion and paraoxon, in order to obtain a proper foundation for evaluating future data regarding the percutaneous absorption of these two important compounds.

Methods

Tissue slices of liver were cut with the Stadie Riggs, 1944, tissue slicer; skin slices were cut as described previously, Fredriksson, 1958. The skin was trimmed carefully to remove excess subcutaneous tissues. The skin of experimental animals was removed immediately after sacrifice, that from humans was obtained from an abdominal area during surgery. None of the patients had a disease which would impair the structural integrity of the skin. The skin was kept in a moist chamber at 5° C for a few hours before testing or kept in the frozen state if longer storage was needed.

The hydrolysis of parathion (E 605, or diethyl 4-nitrophenyl thiononophosphate) or paraoxon (E 600, or diethyl 4-nitrophenyl phosphate) was measured by the Warburg method as described by Augustinsson and Heimburger, 1954 a, 1954 b, or by extraction and semi-quantitative estimation of the *p*-nitrophenol formed after separation on paper. The enzymic reaction was carried out at 25° C in 3 ml of total volume containing 0.0166 M and 0.12 M NaCl, 7.7×10^{-3}M paraoxon, or 7.2×10^{-3}M parathion, and 1 gm of skin slices or 0.1 ml of rabbit plasma in an atmosphere of nitrogen-carbon dioxide 95—5, V/V. The parathion or paraoxon was added with a micropipette to the side arm of the flask along with 0.6 ml of distilled water. After 10 minutes of equilibration at 25° C, the organophosphorus compound was dumped into the main compartment to start the reaction. A control flask that did not contain parathion or paraoxon was run also.

Two other control flasks each of which contained the organophosphorus compound, but not tissue, also were incubated. At the end of the reaction period, tissue was added to one of the flasks and both were acidified and extracted. These latter two flasks served to reveal any non-enzymic hydrolysis of the organophosphorus compound during the incubation period or the extraction procedure.

At the end of the reaction period, 0.2 ml of 2.5 M hydrochloric acid was added to acidify the reaction mixture. The *p*-nitrophenol formed and the remaining organophosphorus compounds were extracted by shaking an aliquot of the reaction mixture 3 times with 4 volumes of ethyl ether each time. The ether extracts were evaporated at room temperature, and the residue was taken up in a 1.0 ml of ethanol. For the isolation of *p*-nitrophenol, the extract was chromatographed at 25° C on Whatman No. 1 paper in n-butanol saturated with 5 M ammonia as described by Erne, 1958. The *p*-nitrophenol, which moved about half way down the paper, readily separated from a large excess of parathion or paraoxon, both of which ran with the front. These compounds were revealed on the paper by spraying with 0.9 N KOH in ethanol and heating at 90° for 5 minutes as described by Karlog, 1957. The amount of *p*-nitrophenol formed was estimated semiquantitatively by comparing the spot size and color intensity with closely matching standards chromatographed at the same time as the extract.

The conversion of parathion to paraoxon in skin was carried out in a manner similar to that described by Davison, 1955, for liver. Skin or liver slices, weighing approximately 1 gm were shaken for 1 or 2 hours at 37° in an oxygenated atmosphere in 6 ml of reaction mixture containing Krebs-Ringer phosphate pH 7.4, 0.01 M glucose, 2.9×10^{-4}M parathion, and 3 percent ethanol.

The reaction mixture in the flask was acidified with 0.2 ml of 2.5 M hydrochloric acid and extracted three times with 1 volume of diethyl ether each time. The residues left after the evaporation of the ether were taken up in ethanol and chromatographed in the ethanol-water-chloroform solvent system on silicone impregnated Whatman No. 1 filter paper described by Metcalf and March, 1953.

The solvent system was modified from that of Metcalf and March by placing the immobile as well as the mobile phase in the chamber. This resulted in a better separation of *p*-nitrophenol and paraoxon. The movement of the paraoxon relative to *p*-nitrophenol is 0.88 in the unmodified solvent (Augustinsson and Jonsson, 1957) and 0.39 in the modified solvent.

Analytical grade parathion and paraoxon were obtained from the American Cyanamide Company. No *p*-nitrophenol could be detected in either compound, and the parathion was free of paraoxon when the respective compounds were chromatographed using a technique that permitted detection of less than 0.2 percent of the impurity.

A sample of parathion labelled with ^{32}P, which was used in other papers of this series, was also uncontaminated with paraoxon or *p*-nitrophenol. However, when autoradiograms were prepared from chromatograms developed in ethanol-chloroform-ammonia by apposition to Eastman Kodak No Screen Medical X-ray film, a faint spot that ran ahead of parathion and behind paraoxon was detected. This impurity could not represent more than 0.1 percent of the radioactivity in the parathion. The nature of the contaminant is unknown, but Augustinsson and Jonsson, 1957, have found that the *bis* (nitrophenyl ester) of ethyl phosphate has about the same R as the impurity in the unmodified solvent of Metcalf and March, 1953.

Results and Discussion

The results of the studies on the hydrolysis of parathion or paraoxon are presented in Table 1. Parathion was not hydrolyzed by the skin of any of the species tested. This organophosphorus compound is very sparingly soluble in water. It was, however, well suspended in the reaction mixture when the reaction vessel was shaken in the water bath in the Warburg apparatus. Attempts to increase the possible rate of hydrolysis of parathion by decreasing the size of the suspended particles of the phosphorus compound with the non-ionic detergents Sterox SE (a polyoxyethylene thioether) at 0.5 percent concentration or Triton X-100 (a monylphenoxy polyethylene oxide ethanol) at 1 percent concentration were unsuccessful even though stable suspensions of parathion were obtained under these conditions, and the hydrolysis of paraoxon by rabbit plasma was inhibited only 50 percent by these detergents. Also, the presence of the latter caused an apparent gas uptake by the skin. This spurious gas uptake may be related to the absorption of water by the skin slices with subsequent change in volume of the tissue. Because of these experimental difficulties, these detergents were not used in any of the other experiments reported in this communication.

As is shown in Table 1, paraoxon was hydrolyzed by slices of rabbit, cat, or human, but not rat skin. The rate was quite slow with human or cat skin. About 1 percent of the initial amount (6325 μg) of paraoxon was converted to *p*-nitrophenol in 90 minutes at 25° by 1 gm of the slices of skin of these two species. This low activity could be detected only by paper chromatography. On the other hand, the rabbit skin had much greater activity; about 20 percent of the initial amount (6325 μg) of paraoxon was hydrolyzed to *p*-nitrophenol under these conditions. With this species, it was possible to determine the activity of the skin slices on the same sample by both the chromatographic and Warburg manometric techniques. As can be seen in Table 1, there was fair agreement between the results obtained by the two methods.

The results of other studies carried out on slices of rabbit skin are presented in Table 2. An enzyme is probably responsible for the ability of this tissue to split paraoxon, since the activity is destroyed by heat and since it was a continuous reaction. Treatment of the rabbit skin before excision with a 0.1 percent solution of the antiseptic, zepharin, a benzalkonium chloride, under the same conditions used for preparation of the skin of patients for surgery, caused only about a 20 percent inhibition of this enzyme. Therefore, the low activity of the human skin that was obtained at operations probably was not a result of preoperative treatment of the skin. As is shown in Table 2, the activity of rabbit skin is readily leached out of the tissue by incubation in the reaction medium. Apparently, the enzyme is in the extracellular fluid or loosely bound to cellular structures. Another possibility is that the enzymic activity is due to the small amount of plasma that may be in the skin. There was, however, no difference in paraoxon splitting activity between bled and non-bled animals. Rabbit plasma does have much more paraoxon-splitting activity than do the other tissues of this animal. Liver was found to have 12 percent and skin only 0.7 percent of the enzymic activity of plasma. Aldridge, 1953, has reported similar relative activities of the liver and plasma of this species.

Table 1. *Hydrolysis of Parathion and Paraoxon by the Skin of Various Species.**

Species	Substrate	Warburg Manometric Method		Chromatographic Method		
		Comments	μM	Comments	μM	Molar Percent Conversion
Man	Paraoxon	No significant differences from skin without paraoxon were noted	—	After 225 min. of incubation; paraoxon blank 0.1 μM	0.7	3
	Parathion	No significant differences from skin without parathion were noted	—	After 225 min. of incubation the *p*-nitrophenol was at same concentration as parathion zero time	—	
Rat	Paraoxon	No significant differences from skin without paraoxon were noted	—	After 90 min. of incubation *p*-nitrophenol was at same concentration as paraoxon zero time	0	0
	Parathion	No significant differences from skin without parathion were noted	—	After 90 min. of incubation *p*-nitrophenol was at same concentration as parathion zero time	—	—
Rabbit	Paraoxon	Gas evolution increased with paraoxon	4.6	After 90 min. reaction time intense spot at position of *p*-nitrophenol	3.4	20
	Parathion	No significant differences from skin without parathion were noted	0	After 90 min. reaction time *p*-nitrophenol spot no darker than in parathion zero time	0	
Cat	Paraoxon	No significant differences from skin without paraoxon were noted	0	After 90 min. of incubation moderately intense spot at position of *p*-nitrophenol	0.2	1
	Parathion	No significant differences from skin without parathion were noted	0	After 90 min. of incubation no different from parathion zero time	0	

* In these experiments, finely minced skin of 1 gm wet weight was used. The dry weights were 29, 40, 36, and 33 percent of the wet weight of the human, rat, rabbit, and cat skin, respectively. The difference in dry weight is probably due to the small amounts of fur left on the skin of the rabbit, rat, or cat. The activity observed in the Warburg procedure was corrected for the non-enzymic hydrolysis of paraoxon and for the evolution of gas in the absence of paraoxon. The chromatographic method was corrected for the non-enzymic hydrolysis of paraoxon or parathion by means of the zero time controls described in the experimental section.

An attempt was made to differentiate between the enzymes present in the plasma and skin by determination of their respective Michaëlis-Menten constants for paraoxon according to the method of Lineweaver and Burk, 1934. It was found that the Michaëlis-Menten constants at 25° C for the paraoxon-splitting enzyme of plasma was 7.7×10^{-4}M and that for the enzyme present in the skin was 6.6×10^{-4}M. Because of the low activity of the skin and the variability of the carbon dioxide evolution from skin alone, the difference between the Michaëlis-Menten constants of these two tissues is probably within

Table 2. *Characteristics of the Factor in Rabbit Skin which Hydrolyzes Paraoxon.**

Experiment Number	Conditions	Activity μM CO$_2$ 90 min.
1	Usual	3.2
	Heated at 80° for 10 minutes	0
2	Usual	3.70
	Skin treated with Zepharin antiseptic	2.90
3	Usual	4.15
	Skin incubated with reaction mixture 60 minutes and then removed before the paraoxon is added from the side arm of the flask	3.75

* In these experiments, 1 gm of rabbit skin was incubated for 90 minutes at 25° C in the Warburg apparatus as described in the experimental section. The observed activity is corrected for the non-enzymic hydrolysis of paraoxon and for evolution of gas from skin in the absence of paraoxon.

the experimental error of measurement of this constant in the skin. The results of these experiments are compatible with the view that the enzyme present in the rabbit plasma and in the skin are the same, but the results are not conclusive evidence since closely related enzymes may have the same Michaëlis-Menten constants. It was not possible to investigate this question with the skin of humans or cats because the activity was too low.

The objective of the next series of experiments was to determine whether the skin of various species contained enzyme systems for the conversion of parathion to paraoxon. Under the conditions used, the formation of paraoxon was readily demonstrated in liver slices as has been noted previously by Gersmann *et al.*, 1952; Gage, 1953; Metcalf and March, 1953; Aldridge, 1955; Davison, 1955; and Kubištova, 1959. With liver after 1 hour at 37° C, about 1 percent of the parathion was converted to paraoxon and another 2 percent was found as *p*-nitrophenol. The phenol probably arose by the hydrolysis of paraoxon, which was produced by the oxidation of parathion rather than by direct hydrolysis of the latter. Accordingly, about 3 percent of the parathion had been converted to paraoxon in 1 hour at 37° C. These findings are in agreement with the previous studies of Davison, 1955, and Kubištova, 1959. Under the same conditions, and even when the reaction period was extended to 2 hours, no evidence of the conversion of parathion to paraoxon by the skin of any of the species tested could be observed on chromatograms of tissue extracts under conditions in which a 0.2 percent conversion could have been detected. Skin slices have less ability than liver to promote the hydrolysis of paraoxon to *p*-nitrophenol. Therefore, paraoxon would have a greater chance to accumulate in the skin than in the liver if any enzyme that catalyzes the conversion of parathion to paraoxon were present. Under these conditions, the failure to detect paraoxon formation by the skin is even stronger evidence that the oxidation of parathion to paraoxon does not take place in this tissue.

The results suggest that parathion can be assumed to be absorbed in essentially unchanged form. Thus, it could be expected that there will be no decrease in its toxicity during the very slow passage through the skin, and this lack of inactivation might make percutaneous absorption of parathion a particularly hazardous portal of entry. Paraoxon, on the other hand, can be hydrolyzed to quite an extent during its percutaneous absorption in three of the species tested. This hydrolysis should result in a relative decrease in the percutaneous toxicity of paraoxon in these species as compared with the rat. It is true that the enzymic activity in the skin is low compared with that of plasma, but percutaneous absorption is a very slow process while transportation of the toxic material within the blood stream is fast. Thus, inactivation within the skin might be of greater toxicologic importance. The results indicate that the skin of man and cat, which showed the lowest activity, was capable of splitting approximately 30 µg paraoxon per hour and gram of tissue at 25° C. This does not represent optimal activity, since the slices used were quite thick, and furthermore, the activity will, of course, be higher at body temperature. What seems to be a low activity might in reality be most important, as the rate of absorption of paraoxon has an order of magnitude of micrograms per hour and cm^2 (Fredriksson, 1961).

SUMMARY

The ability of the skin of man, cat, rabbit, and rat to hydrolyze or otherwise metabolize paraoxon (E 600, or diethyl 4-nitrophenyl phosphate) or parathion (E 605, or diethyl 4-nitrophenyl thiononophosphate) was investigated using the Warburg technique and paper chromatography. Parathion was not hydrolyzed or transformed into paraoxon by the skin of any of the species tested.

Paraoxon, on the other hand, was hydrolyzed by skin from man, cat, and rabbit. This reaction, which was enzymic in nature, occurred at the fastest rate in the rabbit. In this tissue, about 20 percent of the paraoxon was hydrolyzed in 1 hour by 1 gram of skin at 25° C, while only about 1 percent was converted by skin from man or cat.

The relation of these findings to the percutaneous absorption of these compounds was discussed.

RÉSUMÉ

La propriété de la peau de l'homme, du chat, du lapin et du rat d'hydrolyser ou de métaboliser d'une autre manière le paraoxon (E 600 ou diéthyl 4-nitrophényl phosphate) et le parathion (E 605 ou diéthyl 4-nitrophényl thionono-phosphate), a été étudiée au moyen de la technique de Warburg et de la chromatographie sur papier. Le parathion n'a pas été hydrolysé ni transformé en paraoxon dans la peau d'aucune des espèces examinées. Par contre, le paraoxon est hydrolysé par la peau de l'homme, du chat et du lapin. Cette réaction, qui est d'origine enzymatique, s'effectue avec le plus de rapidité chez le lapin. Chez cet animal, le 20 % environ du paraoxon est hydrolysé en une heure par un gramme de peau à 25°, alors que la peau de l'homme et du chat n'en transforme que le 1 % seulement.

L'auteur a discuté la relation existant entre ces résultats et l'absorption percutanée de ces produits.

ZUSAMMENFASSUNG

Die Fähigkeit der Haut von Menschen, Katzen, Kaninchen und Ratten, Paraoxon (E 600, Diäthyl-4-nitrophenyl-phosphat) oder Parathion (E 605, Diäthyl-4-nitrophenyl-thiononophosphat) zu hydrolysieren oder auf andere Weise abzubauen, wurde in der Warburg-Apparatur und mit Hilfe der Papierchromatographie untersucht. Es zeigte sich, dass Parathion in der Haut der untersuchten Spezies nicht in Paraoxon umgewandelt wird.

Andererseits wurde Paraoxon von der Haut von Menschen, Katzen und Kaninchen hydrolisiert. Die Reaktion, die enzymatischer Natur war, verlief am schnellsten bei Kaninchen; ungefähr 20 % des Paraoxons wurden in 1 Stunde durch 1 g Haut bei 25° C hydrolisiert, während die Umsatzrate in der Haut von Menschen und Ratten unter den gleichen Bedingungen nur 1 % betrug.

Die Beziehungen dieser Befunde zur percutanen Resorption dieser Verbindungen wird diskutiert.

RESUMEN

Utilizando la técnica de Warburg y la cromatografía de papel se investigó la capacidad de la piel del hombre, gato, conejo y rata para hidrolizar o metabolizar en otra forma el paraoxon (E 600 o dietil 4-nitrofenil fosfato) o el paration (E 605, o dietil 4-nitrofenil tiononofosfato). Ninguna piel de las especies ensayadas hidrolizó el paration ni lo transformó en paraoxon.

El paraoxon, por otra parte, fué hidrolizado por la piel humana, la de gato y la de conejo. Esta reacción, que fué de naturaleza enzimática, tuvo lugar con la mayor rapidez en el conejo. En éste, aproximadamente el 20 por ciento del paraoxon fué hidrolizado en 1 hora por un gramo de piel a 25° C., mientras que sólo se convirtió un 1 por ciento con piel humana o de gato.

Se discutió la relación de estos datos con la absorción percutánea de estos compuestos.

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TOXICOLOGY OF INSECTICIDES[1]

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Presented at 21st Annual Educational Conference

World War II marked a revolution in the chemical control of insects in agriculture, in public health, and in the household. The importance of insects as pests, as vectors of diseases, and as destroyers of crops had been widely recognized before this time but control measures, both chemical and environmental, were expensive and of only limited effectiveness.

The introduction of DDT to control lice and thus break the chain of transmission of epidemic typhus in troops in Naples, Italy marked the beginning of a new era in control of insect vectors of diseases. This new weapon was rapidly adapted to control of malaria mosquitoes, filth flies, and agricultural pests. DDT was rapidly followed by other chlorinated hydrocarbon compounds such as chlordane, benzene hexachloride, toxaphene, dieldrin, and aldrin.

Very shortly, as a result of a German search for a substitute for nicotine, the organic phosphorus insecticides such as parathion and tetraethyl phyrophosphate were introduced, particularly into agricultural use.

But these new blessings were not without their faults. Enthusiastically the eradication of the housefly by DDT was predicted. Such ill-conceived enthusiasm encouraged the substitution of DDT for sanitation. That this was unwise was promptly demonstrated by the development as early as 1948 of strains of flies resistant to DDT. And so other insecticides had to be substituted for DDT.

This substitution raised new problems. DDT had been determined to be safe for use on humans for louse control and in homes for fly and mosquito control, but how about these newer, more powerful materials? Parathion, although developed as an insecticide, was chemically related to new war gases—was it then safe to use a chemical of such great known toxicity? Already sodium fluoro-acetate or 1080, the powerful new rodenticide, had taken a small toll of children who drank it by mistake.

Of course, toxic insecticides had been used for years. Best known among them were the cyanides, the arsenicals, strychnine, nicotine, and a few others. However, in general these highly dangerous pesticides were well known by the general public as well as to the medical profession. The introduction of a relatively large number of new and unknown pesticides created a more urgent problem, largely because the materials were being introduced faster than it was possible to learn enough about their toxicity to man.

To meet this situation, first the manufacturers of some of the more potent materials such as parathion and 1080 put voluntary restrictions on their sale, designed to prevent their falling into the hands of uninformed laymen. They also conducted and are continuing to conduct educational campaigns to inform the user and others concerned as to safe methods of handling such toxicants.

Secondly, federal and state laws were passed requiring that pesticides must be adequately labelled before they are sold. Such labels must not only identify the active ingredients but directions necessary for safe use must be spelled out on the label together with such other precautionary statements, antidotes, etc. as seem to be justified by the material in question.

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[1] From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Wenatchee, Washington.

Thirdly, the Federal Food and Drug Act was amended to expedite the establishment of tolerances for the amounts of pesticides permitted to remain on raw agricultural products as residues when marketed.

At the same time that these three actions were taking place, a variety of research institutions began or accelerated their program designed to establish the toxicity of these pesticides to man and to devise safe methods of use.

The Toxicology Section of the U. S. Public Health Service, including its Wenatchee Field Station, is one of the few laboratories established for the sole purpose of studying human health hazards involved in the use of these newer pesticides. It has adopted the philosophy that all pesticides are potentially dangerous if misused, but at the same time no pesticide is so toxic that it cannot be safely used for acceptable purposes, if proper precautions are followed. It is the responsibility of every sanitarian and everyone else who uses pesticides to know how to use them safely.

Of course, the first step in safe use of pesticides is the intelligent selection of the proper pesticide and the proper formulation thereof. Basic to this is an appreciation of the principle that there is a cost both in terms of money and labor and in terms of risk to health, in the use of any pesticide. The benefits to be obtained should be greater than the estimated costs. Frequently the use of proper sanitation will cost less and provide more benefits, not only in terms of pest control but in other health measures as well, than will the use of pesticides. In such a situation it would be foolhardy to use a pesticide. However, one cannot overlook potential benefits in public relations. Sometimes, for instance, mosquito control by a fogging machine may be justified as a temporary expedient from a public relations standpoint even if more effective but slower and less spectacular methods are available.

Occasionally one encounters a desire to use newer materials even though the older, and in some instances safer, insecticides might be satisfactory. It has not been uncommon to blame unsatisfactory fly or mosquito control on resistance, when a poorly planned program was at fault.

Other things being equal, the less hazardous insecticide should, of course, be used. Seldom, however, are other things equal. Cost, effectiveness, and persistence are almost certain to vary from one pesticide to another. But if it does come down to hazard, what factors enter into hazard? Leaving out dangers of fire or explosion, factors to be considered include toxicity to man, concentration to be handled, formulation, volatility, persistence, and method and timing of application.

Of these, inherent toxicity to man is perhaps most difficult to evaluate. A common denominator frequently used to compare toxicities is the acute oral LD_{50} to white rats. This is the statistical estimate of the single dosage required to kill fifty percent of a large population. It is usually expressed in terms of milligrams of toxicant per kilogram of body weight of the experimental animal. The toxicity of a compound when consumed for a long period is usually expressed in terms of the concentration (parts per million) of the poison in the food of the animal which is necessary to produce a particular harmful effect. Caution should be used in interpreting these values because of the variability of results from laboratory to laboratory, between sexes, from one species to another, between various formulations, etc. as well as by different routes of entry. Non-fatal effects may be observed during experiments to determine LD_{50}'s or long-term toxicity but are not expressed in the numerical result. Irrespective of the kind of sickness which a given material causes, biochemical, biophysical, and pathological studies must be made if a reasonably good understanding of the mode of action of the poison is to be found.

Although animal experiments necessarily constitute our first source of information about the toxicity of a new compound, information may eventually be obtained about the toxicity to man. Clinical information may be obtained from the careful study of cases of accidental poisoning. These same cases occasionally give useful information on dosage relationships. In some instances very detailed experimental studies have been made. Thus, we have good information on the clinical course of uncomplicated poisoning in man caused by the following newer pesticides:

ORGANIC PHOSPHORUS INSECTICIDES:

TEPP
parathion
paraoxon
demeton
Thimet
malathion

CHLORINATED HYDROCARBON INSECTICIDES:

DDT
BHC including lindane
chlordane
aldrin
dieldrin
endrin
toxaphene

HERBICIDES:

various dinitrophenols

FUNGICIDES:

various organic mercury compounds

RODENTICIDES:

1080
warfarin

The list does not contain compounds such as 2,4-D and ANTU which have apparently not produced uncomplicated poisoning in spite of extensive use. Similar information is, of course, available on the older pesticides and on kerosene and various other solvents. Dosage relationships for man are known for a number of the pesticides. Detailed physiological studies have been made for a few of them, especially DDT and warfarin.

Toxicological and clinical studies may serve to emphasize the extreme hazard of certain materials such as 1080, while emphasizing the high degree of safety of other compounds such as malathion, DDT, and warfarin.

Let us now consider how these various factors would enter into a fly control problem.

If an unknown species of fly has suddenly become a serious problem in your area there might be a temptation to select the most effective insecticide you can find and saturate the area with it by airplane.

The proper procedure would be to identify the species and locate the breeding area. Assuming it was impractical to eliminate the breeding area for one reason or another, the decision to use an insecticide must be preceded by the decision as to whether to apply it to immature stages or to the adult. Generally speaking an insecticide can be applied against the maggots with less hazard because the total area to be treated is apt to be smaller and more specific. If measures must be directed against the adult, can the insects be reached better by some form of space spray or by a residual application to their resting places? The latter is apt to be safer because of the more limited areas to be treated.

Let us assume a residual spray against either larvae or adults is practical. Probably the first insecticide that should be considered is DDT. Is it effective against the species concerned? Has this particular strain developed resistance to DDT? If DDT is not effective which residual insecticides are? Can the surface be treated without contaminating food, water, dishes, etc.? Is the insecticide sufficiently volatile that the fumes might be dangerous to people working or living in the treated area? Will the use of spot treatments or the hanging of treated cords or the use of poison baits be satisfactory? If so, more toxic materials including some of the organic phosphorus compounds might be safe.

If space treatment is necessary to kill adults, can the application be made when no one is in the space to be treated? Will the toxicant be gone from the air before the space is re-occupied? Continuous vaporizers using such materials as lindane present a needless hazard in sleeping quarters or in kitchens and elsewhere where human food may be contaminated.

Experiments have shown for instance that lindane vapor produced by electric vaporizers can penetrate into packaged foodstuffs such as breakfast cereals. Also, the lindane will, under certain circumstances, condense on solid surfaces and then revolatilize so that it becomes quite difficult to predict what the concentration of lindane will be under all circumstances. Lindane has produced convulsions in humans, and in several cases when children have consumed portions of the pellets designed for use in vaporizers they have been made seriously ill. Since other methods of fly control, such as parathion treated cords are more effective without producing hazards of continuous exposure to vapor or easy availability to children, there seems to be little reason for using the vaporizers.

With any type of treatment the operator should take reasonable precautions to prevent excessive exposure to himself. In a tightly closed space a full-face gas mask may be necessary, whereas a half mask respirator may be adequate under more open conditions and in the open. Less toxic and less volatile materials may not require any respirator. Caution should be used in selecting a respirator for some cartridges are recommended for one material such as parathion but not for the closely related tetraethyl pyrophosphate. Proper fitting and freqent replacement of cartridge and filters are essential or respirators may produce a false sense of security.

Protective clothing may or may not be necessary depending upon the material and the conditions of application, but personal hygiene in terms of thorough washing before eating, drinking, or smoking, daily bathing, and daily laundering of clothing is essential in any case. It has been shown in one instance that a man wearing gauntlet rubber gloves was getting more exposure to his hands inside his gloves than he would have had without gloves at that particular time. He had simply donned the gloves while his hands were contaminated and thus contaminated the inside of his gloves. Thereafter putting on those gloves was no protection.

Although the procedures used for the control of disease vectors in this country are safe, sooner or la[illegible] the sanitarian is apt to run into suspected poisoning cases associated with accidental or agricultural exposure. What is his responsibility when such cases come to his attention?

In frank poisoning by the more toxic organic phosphorous compounds the patient may stop breathing within a matter of minutes after the first signs of poisoning are noted. In any such case of acute poisoning immediate medical attention is obviously of prime importance. However, simply referring the patient to a doctor is not always enough. Steps should be taken to make sure the patient is still breathing when the doctor first sees him. This may well require artificial respiration in the interim and this takes precedence over all other considerations if the patient requires it. One more step that the sanitarian should take is to urge the patient or responsible individual to take the pesticide container to the doctor. The label will not only identify the active ingredient but with the highly toxic compounds the label provides the doctor with useful information on antidotes.

One more step may be justified particularly if there is an unavoidable delay in reaching a doctor. In such cases the sanitarian may be justified in calling the nearest poison control center for aid in identifying the toxicant if it is unknown and for advice on first aid. With the growing number of poison control centers throughout the country, every sanitarian should know where to locate the nearest one and something about their policies, for they vary from center to center. In some localities the sanitarian may even be involved, usually sharing the responsibility with a nurse, in investigating the circumstances surrounding accidental poisoning cases. If so, he should be properly trained in his duties by the person assigning the job to him. It might not even be amiss for a sanitarian to know that we in Wenatchee and our headquarters in Savannah, Georgia are available for consultation to physicians in emergencies involving the newer pesticides. These calls supplement our "Clinical Memoranda on Economic Poisons" which have been widely circulated among physicians who might reasonably expect to treat poison cases. Copies of the Memoranda can be obtained from the Government Printing Office in Washington, D. C. at a small cost.

So in summary, sanitarians should know that the use of pesticides may present some hazard and this is only one of many reasons why engineering or environmental methods of insect control should be favored if practical. However, they should also realize that any recommended insecticide can be safely used if label directions including good personal hygiene are followed. In spite of all, accidental poisonings will occasionally occur and the sanitarian can be useful in such cases if he understands basic first-aid including artificial respiration and if he knows where technical consultation is available.

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Review of the Toxicity of Hexachlorophene, Including Its Neurotoxicity

PESTICIDE
PUBLICATION

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THE toxicity of hexachlorophene has recently been reviewed.[1,2] Poisoning in humans is usually due either to accidental ingestion because hexachlorophene is mistaken for milk of magnesia or to application of hexachlorophene to large areas of burned or otherwise damaged skin. Larson et al.[3] indicated that the incidence of convulsions in burned patients decreased when the use of hexachlorophene was discontinued. Hexachlorophene poisoning may play a role in the etiology of burn encephalopathy. Symptoms of poisoning and outcome of the published cases cited in the two reviews are given in Table I. Additional human cases of hexachlorophene poisoning have been reported more recently.[4-6] The cases reported by Mullick consisted of four children with dermal exposure and two adults who had accidentally ingested 3% hexachlorophene. Treatments with 3% hexachlorophene baths were given to two of the children for burns and to two of the children for severe ichthyosis. All patients died, and microscopic examination showed severe vacuolization of the white matter of the brains of the children, but not of the two adults. Korlof and Winsten reported three cases; two of these were burn cases that developed central and peripheral nerve damage and renal disturbance thought to be due to absorption of 3% hexachlorophene dermally applied. The third case showed signs of kidney damage after ingestion of the material.

Aside from the systemic toxicity, hexachlorophene may also cause skin irritation and other local reactions in sensitive individuals.[7-9]

The acute toxicity in animals varied with the animal species and the route of administration. In our Sherman strain rats, the single oral LD_{50} for adult male rats was 66 mg/kg and for adult female rats 56 mg/kg; for weanlings it was 120 mg/kg when the material was given in peanut oil by stomach tube.[10] A lower dietary level of 20 ppm (2.3-1.1 mg/kg/day) did not affect reproduction in this study. The acute intravenous LD_{50} was 7.5 mg/kg in adult male rats. Single oral doses of 40 to 50 mg/kg were lethal to dogs.[1] The oral LD_{50} for sheep and cattle ranged between 30 and 60 mg/kg.[11]

We conducted a two-generation rat reproduction study in our laboratory and found a reduced survival of the offspring of the F_1 generation fed 100 ppm (11.8-5.5 mg/kg/day) in their diet for 54 and 166 days. This was statistically significant in the F_{1b} generation. In the F_2 generation, the litters tended to be smaller.[10] There was some evidence that doses toxic to the dam caused malformations in the offspring.[12,13]

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6

TABLE I

Toxic Reaction in Humans after Hexachlorophene Exposure*
(Published cases)

No. cases	Type of exposure	No. of patients with GI sympt.	No. pts. with periph. circ. failure, body temp. fluctuation	No. pts. with CNS sympt.	Outcome of patients
11 (1 child 10 adult)	Single oral dose, 3% in detergent	10	3	1	1 death 9 recoveries 1 ?
1 (infant)	Oral, 37 mg/kg/day × 7	1	1	1	Recovery within 2 m
	Oral,				
60	20 mg/kg/day × 1	50–66%	7	13 (headache)	Recovery in few days
22	20 mg/kg/day × 2	50–66%	7	13 (headache)	
8 (5–15 yrs)	20 mg/kg/day × 3	50–66%	8	1 (convl.)	
6 (3 child 3 adult)	3% in detergent dermal, repeated, burned patients	1		5 1 (diplopia)	Recovery
1	3% in detergent, intact skin, used as lotion after bath for 4 days			1	Recovery within 16 days

* Accidental ingestion or application on burned and in one instance on intact skin.

Repeated application of hexachlorophene in either a detergent or propylene glycol solution to the shaved back of rats caused skin irritation which could become quite severe and also caused systemic toxicity. It was not known how much the skin lesions affect percutaneous absorption.[10] Carroll et al.[14] exposed the tails of rats to radioactive hexachlorophene and found that small amounts of hexachlorophene were absorbed through the intact tail skin of the rat. Immediately after the development of a second degree burn, the average absorption rate was 2½ times that of the normal skin surface. Small amounts of extractable hexachlorophene were also absorbed through intact human skin. When ten adult volunteers bathed daily for 10 minutes with heavy lathering using a 0.75% hexachlorophene cake soap for 21 days, the mean HCP blood level on the 21st day was 0.52 μg/ml for males and 0.28 μg/ml for females.[4] The mean pre-exposure blood level for males was 0.009 μg/ml and for females 0.007 μg/ml.[15]

A single oral dose of 100 mg/kg hexachlorophene produced diarrhea and leg weakness in the weanling rat. Adult rats developed leg weakness after continuous exposure to hexachlorophene of 12 to 19 days at a dietary level of 500 ppm (30 to 18 mg/kg/day). Neurological symptoms were not produced at a dietary level of 100 ppm. Other species which also show neurological deficits after exposure to hexachlorophene were rabbits,[16] pigs,[17] and cats.[18] Sheep became blind, according to Udall and Malone.[11] Hanig et al.[18] recorded the neurological deficits produced in cats by hexachlorophene in more detail. Early symptoms consisted of lassitude, weakness and ataxia of hindlimbs, impaired righting reflex, patellar hyperreflexia which later developed into hyporeflexia, urinary retention, and eventually complete flaccid paralysis. The cranial nerve function remained intact in cats except preterminally. These cats also showed an increase in the cerebrospinal fluid (cisternal) pressure.

The morphological changes produced in the brain have been described.[19] The wet weight of the brain increased, particularly when rats were exposed to hexachlorophene over a period of time.[20] This increase in wet weight was accompanied by an increase in sodium and chloride in the brain, while potassium was only slightly increased in early exposure.[21]

At autopsy, if exposure to high doses of hexachlorophene had continued for some time the brain was soft and gelatinous on gross inspection, and it was difficult to remove the brain from the cranial cavity without injuring it. Microscopic examination of formalin- or Bouin-fixed brain tissue showed normal gray matter and status spongiosus of the white matter (Figs. 1 and 2). The white matter had expanded and consisted of smaller and larger vacuoles lined by strands of eosinophil material which stained positive for myelin. The vacuoles appeared empty. At a dietary hexachlorophene level of 400 or 500 ppm fed over two or more weeks, this vacuolization extended over the entire white matter of the brain and spinal cord. The other two dietary levels used in these studies were 100 ppm and 20 ppm.[10] The dietary level of 20 ppm had no effect on the brain. At the dietary level of 100 ppm, focal areas of vacuolization of the white matter of the brain were observed in some animals, but they were never extensive. The rats on the lower dietary levels did not show neurological deficits. Brain lesions could not be produced in the adult rats with a single dose of hexachlorophene. However, when a single oral dose of 100 mg/kg was given to weanling female rats, mild status spongiosus of the white matter was seen in the brain on microscopic examination.

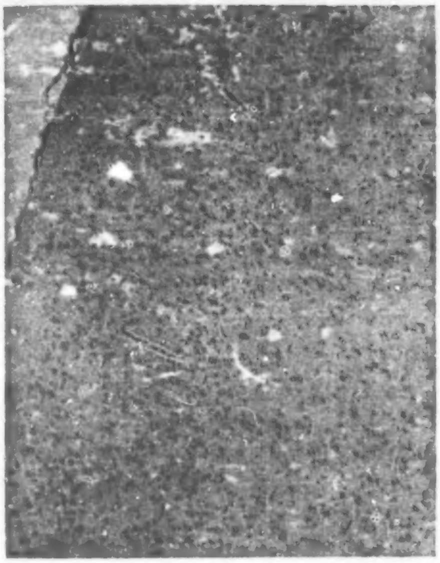

Fig. 1. Section of normal rat brain. Hematoxylin and eosin stain × 100.

On electron microscopic examination of sections from different areas of the brain, the gray matter was normal while the white matter showed large vacuoles that were lined by portions of myelin sheath (Fig. 3). Occasional vacuoles contained electron-dense granules or dark fiber strands. Closer examination of the vacuoles with the electron microscope showed that the vacuoles were due to a split in the interperiod line of the myelin seat. On extensive examination of the brains with the electron microscope, the gray matter was completely normal. After rats were fed 500 ppm of hexachlorophene for a period of ten weeks and then exposure discontinued, the animals gradually recovered and the lesions became less pronounced in the brain.[19]

Monkeys have also shown this lesion.

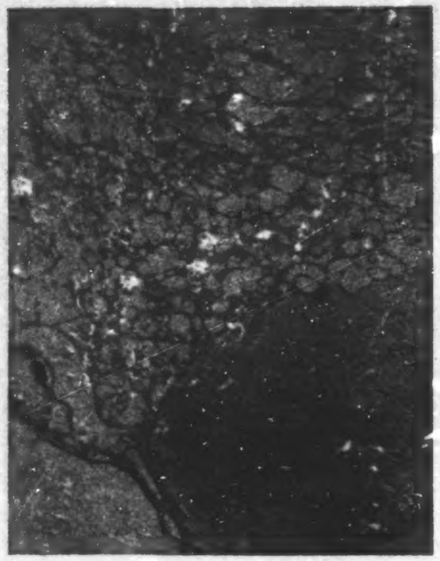

Fig. 2. Section of rat brain showing the vacuolization of the white matter. Hematoxylin and eosin stain×100.

Newborn monkeys washed with a 3% hexachlorophene detergent solution developed a diffuse status spongiosus of the white matter.[22] Santolucito[23,24] was able to produce the vacuolization of the white matter, referred to above as status spongiosus, by injecting adult squirrel monkeys daily subcutaneously with 5 mg/kg of hexachlorophene for 37 days. The lesion was also produced in sheep.[25]

Other chemicals also produce status spongiosus of the white matter of the central nervous system in various species.[26-28]

It has been established[29,30] that small amounts of hexachlorophene penetrate the intact skin and can be identified in blood, adipose tissue, brain, and other organs.

Rats fed 500 ppm hexachlorophene in their diet, had pronounced brain lesions and showed mean blood levels of 8.5 ppm (μg/ml), and those fed 100 ppm showed mean blood levels of 1.21 ppm. Blood levels found in the adult general population and in infants washed with hexachlorophene were below these values (Table II). Fifteen premature infants, 1 to 54 days old, that were washed with 3% hexachlorophene detergent solution, had average extractable hexachlorophene blood levels of 0.475 ppm (μg/Gm) with a range of 0 to 1.6 ppm.[31]

When interpreting blood levels, it is important to realize that the recovery rate varies depending upon the analytical method used to determine hexachlorophene.[32] Presently it is assumed that plasma levels are about twice as high as whole blood levels, although a comparative study has thus far not been published. In rats, after a single oral dose of 50 mg/kg ^{14}C-labeled hexachlorophene, a peak blood level was reached in 24 hours, then it dropped to half within the

TABLE II

Hexachlorophene (HCP) Concentration (PPM in Blood)*
(ppm = μg HCP/Gm of whole blood)

	Infant		Adult
	Cord blood	Discharge from Hospital	General Population
	(N=50)	(N=50)	(N=14)
Range	0.003–0.182	0.009–0.646	<0.001–0.089
x	0.022	0.109	0.028
S.E. ±	0.005	0.016	0.008

* Reprinted from *Pediatrics* 51:392 (1973) with permission from the American Academy of Pediatrics.

Fig. 3. Electron micrograph showing the split in the myelin sheath of the white matter of the brain. Lead citrate and uranyl acetate × *20,900.*

next three days and thereafter gradually leveled off in the next two weeks.[33] This blood-level curve may vary in different species and with different routes of administration of hexachlorophene. After repeated dermal application in humans at low concentration, a residue on the skin resulted in a constant blood level after three or more days of applications. In a human fatal poisoning, the hexachlorophene blood level obtained between 24 and 48 hours following the last topical exposure to the chemical was 2.2 μg/ml (ppm).[14]

In addition to the neurotoxic effects, hexachlorophene was found to be a very potent uncoupler of oxidative phosphorylation.[34-36] This effect was responsible for the body temperature elevation that these authors observed in rats poisoned with hexachlorophene, as well as for the pronounced rigor mortis and rapid onset of rigor mortis when rats that have been given hexachlorophene were sacrificed.

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